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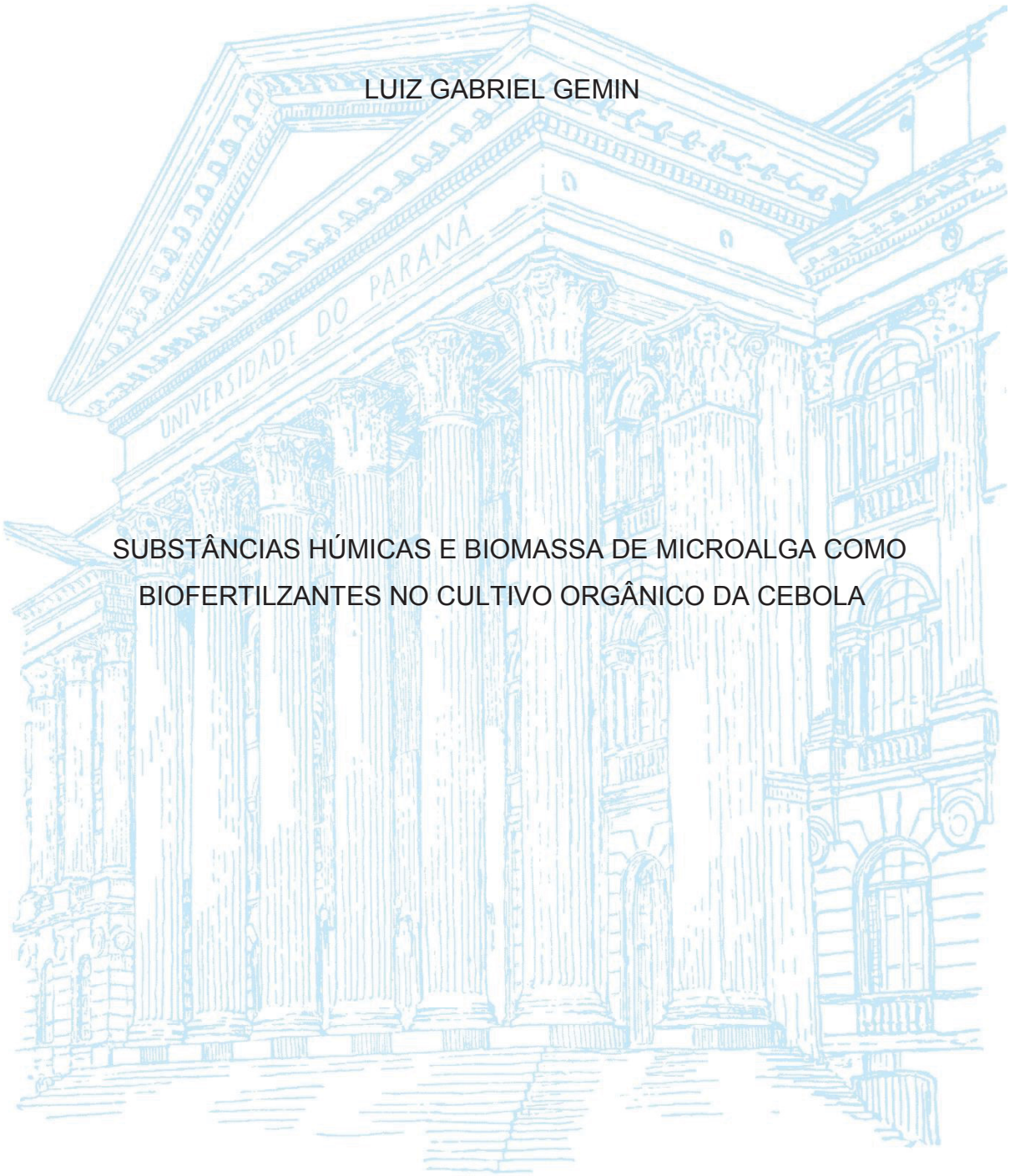
LUIZ GABRIEL GEMIN



SUBSTÂNCIAS HÚMICAS E BIOMASSA DE MICROALGA COMO BIOFERTILZANTES NO CULTIVO ORGÂNICO DA CEBOLA

CURITIBA

2020

LUIZ GABRIEL GEMIN

SUBSTÂNCIAS HÚMICAS E BIOMASSA DE MICROALGA COMO BIOFERTILZANTES NO CULTIVO ORGÂNICO DA CEBOLA

Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanidade, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. Átila Francisco Mógor

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **LUIZ GABRIEL GEMIN** intitulada: **SUBSTÂNCIA HÚMICAS E BIOMASSA DE MICROALGA COMO BIOFERTILIZANTES NO CULTIVO ORGÂNICO DA CEBOLA**, sob orientação do Prof. Dr. ÁTILA FRANCISCO MÓGOR, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 28 de Fevereiro de 2020.

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“*Pensar é o trabalho mais difícil que existe, e esta é provavelmente a razão por que tão poucos se dedicam a ele*.”

Henry Ford

RESUMO

O potencial agrícola das microalgas tem despertado interesse, principalmente devido à identificação de substâncias que são sintetizadas por estes organismos, tais como aminoácidos, proteínas, carboidratos e fitohormônios, que aplicados em plantas promovem o desenvolvimento e crescimento das partes aérea e radicular. As substâncias húmicas, dentre estas o ácido húmico e fúlvico, também apresentam efeito de promoção do crescimento vegetal, sendo o estímulo ao crescimento das raízes o mais pesquisado. Deste modo, com a finalidade de estudar o potencial da aplicação da microalga *Scenedesmus subspicatus* (Sc) em associação com substâncias húmicas no cultivo orgânico de duas cultivares de cebola (BR-29 e Perfecta F1), foram instalados experimentos na área de Olericultura Orgânica da Universidade Federal do Paraná (UFPR). As cepas de Sc foram produzidas em cultivo axênico autotrófico semi-contínuo em fotobiorreatores utilizanto meio de cultura WC no Departamento de Fitotecnia e Fitossanidade da UFPR e as frações utilizadas das substâncias húmicas foram o ácido húmico (HA) e fúlvico (FA) foram obtidos por extração do mineral leonardita. As etapas da pesquisa são apresentadas em quatro capítulos. Inicialmente, o objetivo foi verificar o efeito do HA e da associação do HA+Sc por meio de bioensaios utilizando a planta modelo *Vigna radiata*, cuja finalidade foi determinar as melhores concentrações e combinações a serem utilizados em plantas de cebola. Com base nos resultados dos bioensaios, testou-se a aplicação via imersão das raízes de duas cultivares de cebola no momento do transplantio, em soluções contendo HA+Sc, posteriormente cultivadas em vasos e a campo no sistema orgânico. Foram analisadas as variáveis biométricas no desenvolvimento inicial das plantas em vasos. Na sequência, quantificou-se no campo a produtividade, concentração de macro e micronutriente, bem como alterações bioquímicas nos bulbos. No segundo capítulo, avaliou-se a influência dos tratamentos HA+Sc via imersão de raízes de plantas cultivadas no campo, no armazenamento de duas cultivares de cebola, aferindo a perda de massa e alterações bioquímicas dos bulbos. No terceiro capítulo verificou-se a bioatividade do FA, da Sc e combinação (FS) através de bioensaio. Após determinar as melhores concentrações os tratamentos consistiram em aplicações foliares em diferentes cultivares de cebola em vaso e a campo. Posteriormente, analisaram-se as variáveis biométricas de plantas em vaso e a campo, produtividade de bulbos, alterações bioquímicas e influência dos tratamentos no armazenamento de bulbos. Por fim, no quarto capítulo avaliou-se a capacidade antioxidante dos tratamentos contendo HA+Sc via imersão de raízes em bulbos de duas cultivares de cebola cultivadas no campo. Como resultados, a aplicação de HA+Sc por imersão de raízes promoveu o crescimento vegetal em plantas de cebola, proporcionando ganhos na produtividade e concentração de açúcares nos bulbos, com redução da perda de massa no armazenamento e aumento na capacidade antioxidante em bulbos da cultivar de polinização aberta. Além disso, a aplicação via foliar do FA, da Sc e FS proporcionaram aumento da parte aérea de plantas em vaso, incrementando a produtividade e concentração de açúcares nos bulbos, refletindo em menores perdas de massa durante o armazenamento. Desta forma, conclui-se que a aplicação de substâncias húmicas (HA e FA) e da Sc no cultivo orgânico de cebola apresenta potencial biofertilizante.

Palavras-chave: *Allium cepa* L.*;* Ácido Húmico; Ácido Fúlvico; Agricultura Orgânica, Crescimento vegetal.

ABSTRACT

The agricultural potential of microalgae has aroused interest, mainly due to the identification of substances that are synthesized by these organisms, such as amino acids, proteins, carbohydrates and phytohormones, which applied in plants promote the development and growth of the aerial and root parts. The humic substances, among these humic acid and fulvic also have the effect of promoting plant growth and stimulating growth of the roots. Thus, in order to study the potential application of the microalga *Scenedesmus subspicatus* (Sc) in combination with humic substances in the organic cultivation of two onion cultivars (BR-29 and Perfecta F1), experiments were installed in Organic Vegetable Crops Area Federal University of Paraná (UFPR). The Sc strains were grown in autotrophic axenic semi-continuous cultivation system in a photobioreactor using WC culture medium maintained at constant temperature and light, at the Plant Science and Crop Protection Department of the Federal University of Paraná and fractions used humic substances were humic acid (HA) and fulvic acids (FA) were obtained by extraction of leonardite mineral. The research steps are presented in four chapters. Initially, the objective was to verify the effect of HA and the association of HA + Sc by bioassays using the *Vigna radiata* model plant, whose purpose was to determine the best concentrations and combinations to be used in onion plants. Based on the results of bioassays, the application was tested by immersion of two onion root cultivars at the time of transplanting, in solutions containing HA + Sc subsequently grown in pots and in the organic field. Biometric variables were analyzed in the initial development of potted plants. In the sequence, the yield, macro and micronutrient concentration were quantified in the field, as well as biochemical changes in the bulbs. In the second chapter, we evaluated the influence of HA + Sc treatments by immersing roots of field grown plants in the storage of two onion cultivars, measuring the mass loss and biochemical changes of the bulbs. In the third chapter examined the bioactivity of FA, SC and combination (FS) by bioassay. After determining the best concentrations the treatments consisted of foliar applications in different potted and field onion cultivars. Subsequently, the biometric variables of potted and field plants, bulb yield, biochemical changes and influence of treatments on bulb storage were analyzed. Finally, in the fourth chapter, the antioxidant capacity of treatments containing HA + Sc was evaluated via root immersion in bulbs of two onion cultivars grown in the field. As a result, the application of HA + Sc by root immersion promoted plant growth in onion plants, providing gains in productivity and sugar concentration in the bulbs, with reduction of mass loss in storage and increase in antioxidant capacity in open pollinated cultivar bulbs. In addition, the foliar application of FA, Sc and FS provided increase in the aerial part of potted plants, increasing the yield and sugar concentration in the bulbs, reflecting in lower mass losses during storage. Thus, it is concluded that the application of humic substances (HA and FA) and Sc in the organic cultivation of onions has biofertilizer potential.

Keywords: *Allium cepa* L.*;* Humic Acid; Fulvic Acid; Organic Farming; Plant Growth.

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SUMÁRIO

1. INTRODUÇÃO GERAL

A geração de novos conhecimentos interligado a uma perspectiva conservacionista e eficiente na produção de alimentos, mostra-se um desafio na agricultura moderna a ser vencido por parte dos pesquisadores. Neste sentido, o desenvolvimento de tecnologias sustentáveis que atendam em paralelo a demanda de produtores rurais e consumidores torna-se primordial.

O uso de biofertilizantes em sistemas alternativos de produção, como agricultura orgânica, apresenta-se como uma ferramenta que propicia aumento na produtividade, bem como incremento de aspectos nutricionais dos cultivos (DU JARDIN, 2015). Segundo Yakhin et al. (2017) o conceito de biofertilizantes na ciência de plantas é definido como produtos cujas matérias primas são fontes naturais, utilizados em pequenas concentrações, com objetivo de melhorar processos fisiológicos e bioquímicos, aumentando o potencial genético e produtivo das plantas, devido a alterações no estado hormonal, ativação de processos metabólicos, aumento de aspectos da nutrição, crescimento, desenvolvimento e fortalecimento de plantas reduzindo efeitos negativos de estresse.

São considerados como fontes biofertilizantes as substâncias húmicas, hidrolisados proteicos, extratos de algas e microrganismos benéficos, tais como fungos e bactérias (DU JARDIN, 2015). Recentemente o uso de microalgas vem sendo explorado e mostra potencial na promoção do crescimento vegetal com aumento na produtividade em espécies de importância para horticultura (MÓGOR et al., 2018; GARCIA-GONZALEZ; SOMMERFELD, 2016).

Além da promoção do crescimento vegetal propiciado pelo uso de microalgas em cultivos, outros resultados benéficos no metabolismo primário das plantas vem sendo identificado como a biofortificação em vegetais. A aplicação de *Clorella vulgaris* e *Spirulina platensis* promoveram o enriquecimento dos açúcares totais, aminoácidos e compostos fenólicos (antioxidantes) em plantas de cebola (DINESHKUMAR et al., 2018). Além disso, a aplicação da microalga *Dunaliella salina* elevou a concentração de compostos relacionados a redução do estresse oxidativo em tomateiro sob efeito de salinidade (EL ARROUSSI et al., 2018).

As substancias húmicas, tais como o ácido húmico e fúlvico, são biofertilizantes de grande potencial para uso na agricultura, pois apresentam capacidade de atuar positivamente no metabolismo das plantas ocasionando o crescimento e desenvolvimento vegetal (CANELLAS et al., 2015). Além disso, estudos mostram que

a aplicação de substâncias húmicas em plantas, podem ser consideradas técnicas de biofortificação agronômica, pois apresentam potencial em incrementar sua composição mineral e de alterar positivamente a concentração de açúcares totais, aminoácidos, proteínas e compostos fenólicos (BILLARD et al, 2014; CONSELVAN et al., 2017). O efeito mais conhecido da aplicação de substâncias húmicas é o de modificar a arquitetura das raízes, pois quando absorvida pelas plantas, aumentam a atividade da enzima H+ATPase proporcionando divisão celular, efeito semelhante ao fitohormônio auxina (CANELLAS; OLIVARES, 2014).

Pesquisas com aplicação de microalgas e substâncias húmicas em plantas geraram avanços na agricultura, elucidando os efeitos benéficos destas fontes como biofertilizantes. Entretanto, a sinergia entre essas duas substâncias ainda é inexplorada. Deste modo, foi investigado nesta pesquisa os efeitos da aplicação da microalga *Scenedesmus subspicatus* (Sc) em associação a substâncias húmicas (ácido fúlvico e húmico) no cultivo de cebola orgânica em quatro etapas, apresentadas em forma de capítulos.

Inicialmente testou-se a bioatividade de soluções contendo a biomassa da microalga (Sc), do ácido húmico (HA) e de suas combinações (HA + Sc) por meio de bioensaios com plantas teste (*Vigna radiata* L.). Na sequência, foi realizada a aplicação via imersão das raízes das mudas de cebola em soluções contendo HA+Sc, identificando a influência da aplicação no crescimento inicial em vasos por 60 dias e a campo até o final do ciclo do cultivo, aferindo as variáveis biométricas, nutricionais e bioquímicas dos bulbos (capítulo 1).

No segundo capítulo identificou-se a influência do tratamentos (HA+Sc) via imersão de raízes no armazenamento dos bulbos por um período de 60 dias em ambiente ventilado. Foram aferidas as massas quinzenalmente e realizadas análises bioquímicas nos bulbos após o período de armazenagem.

No terceiro capítulo verificou-se a influência da aplicação de ácido fúlvico (FA), da microalga *Scenedesmus subispicatus*, identificada no terceiro capítulo como SC, e a associação de FA e SC aplicados via foliar em duas cultivares de cebola. Para tanto, foram realizados bioensaios com plantas teste (*Vigna radiata* L.) para verificar a bioatividade e a melhor concentração de FA, SC e FA+SC. Na sequência, foram realizadas aplicações foliares de FA, SC e combinação em duas cultivares de cebola plantadas em vaso conduzidas por 60 dias, avaliando-se as alterações no crescimento da parte aérea. A campo foi realizada a aplicação foliar dos mesmos tratamentos do

experimento em vaso, aferindo-se as variáveis biométricas, bioquímicas e alterações das massas dos bulbos armazenados por 60 dias.

No quarto capítulo identificou-se a influência do tratamentos (HA+Sc) via imersão de raízes na biofortificação e na capacidade antioxidante de duas cultivares de cebola conduzidas em sistema orgânico.

2. REVISÃO DE LITERATURA

2.1 Espécie e cultivo da cebola

A localização geográfica que indica, em sua maioria, a origem do gênero *Allium,* compreende a região holoártico a partir do subtrópicos secos da zona boreal, sendo as regiões do Mediterrâneo, Ásia Central e Paquistão detentora da maior diversidade de espécies deste gênero (FRITSCH; FRIESEN, 2002). A cebola (*Allium cepa* L.) pertencente à família das Alliaceae, historicamente apresenta como centro de domesticação a região montanhosa da Ásia central que inclui o Turcomenistão, Uzbequistão, Norte do Irã, Afeganistão e Paquistão (SOUZA; ASSIS; ARAÚJO, 2015).

Segundo dados da *Food and Agriculture Organization* (FAO, 2017) a produção mundial de cebolas de 2010 (cerca de 79 milhões de toneladas) a 2017 (97 milhões de toneladas) cresceu cerca de 22%. A China é o maior produtor (26% da produção mundial) e o Brasil é ocupante do 9º lugar no ranking. Dentre os países da América do Sul, o Brasil se destaca no volume total, com 1,6 milhões de toneladas de bulbos colhidos, em uma área de 51,9 mil hectares, resultando em uma produtividade de 31 toneladas por hectare (FAO, 2017).

Dada a importância da cebolicultura no Brasil, a atividade encontra-se em 3º lugar no ranking em importância econômica entre as hortaliças, sendo superada pela produção de batata e tomate (KURTZ et al. 2013). A região brasileira com maior destaque no volume de produção de bulbos de cebola é a Sul, sendo o estado de Santa Catarina o maior produtor (431 mil toneladas), seguido do Rio Grande do Sul (175 mil toneladas) e Paraná (133 mil toneladas) (IBGE, 2017).

A espécie *A. cepa* L. morfologicamente é uma planta herbácea, com folhas simples, incompletas, subuladas, invaginantes e constituídas de duas partes distintas: bainha (fechada) e o limbo (com seção transversal cilíndrica). As bainhas das folhas mais velhas formam a casca e protegem as bainhas das folhas mais internas e dos primórdios foliares que se sobrepõe e acumulam reservas, formando o bulbo tunicado (SOUZA, ASSIS & ARAÚJO, 2015).

A quantidade de horas de luz (fotoperíodo) e temperatura adequada são fatores determinantes para a bulbificação da cebola, sendo que estas variáveis dependem das exigências fisiológicas de cada cultivar. Plantas de dias curtos necessitam de um fotoperíodo de 10 horas de luz, as intermediárias de 12 a 14 horas de luz e de dias longos superior a 14 horas de luz, sendo as cultivares de dias intermediários mais

indicadas para plantio na região Sul do Brasil (LEITE, 2014). As plantas de cebola suportam temperaturas baixas no início do desenvolvimento vegetativo (<15ºC), contudo, em fase mais avançada do crescimentos das plantas, temperaturas mais baixas induzem ao florescimento prematuro (Bolting) e em temperaturas altas (>35ºC) podem provocar a bulbificação precoce, ficando estas sem valor comercial (OLIVEIRA & BOITEUX, 2003). A relação fotoperíodo e temperaturas acima do mínimo necessário possibilitará a mobilização de reservas da planta, tendo como consequência a formação do bulbo (LEITE, 2014).

A base da produção brasileira de cebola utiliza cultivares de polinização aberta (OP) (cerca de 80%), do tipo 'Baia Periforme' e 'Crioula'. Essas cultivares dominam o mercado, pois possuem tolerâncias a doenças, apresentam boa conservação pós-colheita e aceitação no mercado (SANTOS et al. 2013). Cebolas do grupo 'Crioula' apresentam adaptação na Região Sul, sendo responsáveis pelo desenvolvimento alcançado pela cultura em Santa Catarina (LEITE, 2005). Por outro lado, cultivares híbridas vem ganhando interesse pelos cebolicultores pois, possuem maior homogeneidade genética quando comparado a indivíduos de OP, são mais adaptadas a plantios adensados e alcançam maiores produtividades (SOUZA; ASSIS &; ARAÚJO, 2015).

Após a colheita dos bulbos de cebola, a armazenagem caracteriza-se por uma etapa muito importante no sistema produtivo. Nesta fase, ocorrem perdas por doenças, brotação, enraizamento, sendo a perda de massa a mais evidente (LIMA & RESENDE, 2007). Fatores como cultivar, práticas agronômicas e condições ambientais são fundamentais para amenizar perdas no armazenamento dos bulbos. (SOUZA; ASSIS &; ARAÚJO, 2015). Além disso, técnicas que favoreçam o aumento de matéria seca e açúcares nos bulbos de cebolas resultam durante o armazenamento, redução na perda de massa (KAHSAY et al. 2013).

2.2 Agricultura orgânica

A agricultura orgânica tem como preceito a utilização de práticas que enfatizem o manejo sustentável de ecossistemas, sistemas integrados de cultivo e pecuária, com uma produção diversificada, empregando práticas naturais no controle de pragas e doenças, livres de tratamentos químicos convencionais (SUCIU et al. 2018). Neste sentido, no Brasil, a Lei nº 10.831, de dezembro de 2003, regulamentada pelo decreto

nº 6.323, de 27 de dezembro de 2007, dispõe sobre a agricultura orgânica, dá outras providencias e estabelece a seguinte conceituação e definição oficial:

> Considera-se sistema orgânico agropecuária todo aquele em que se adotam técnicas especificas, mediante a otimização do uso de recursos naturais e socioeconômicos disponíveis e o respeito à integridade cultural das comunidades rurais, tendo por objetivo à sustentabilidade econômica e ecológica, a maximização dos benefícios sociais, a minimização da dependência de energia não-renovável, empregando, sempre que possível, métodos culturais, biológicos e mecânicos, em contraposição ao uso de materiais sintéticos, a eliminação do uso de organismos geneticamente modificados e radiações ionizantes, em qualquer fase do processo de produção, processamento, armazenamento, distribuição e comercialização, e a proteção do meio ambiente.

No Brasil a agricultura orgânica é adotada em sua maioria por agricultores de cunho familiar (LOURENÇO et al. 2017). De acordo com o Instituto Brasileiro de Geografia e Estatística (IBGE), a região com maior número de estabelecimentos agropecuários com produção orgânica é a Nordeste, seguidos pelas regiões Sul e Sudeste (IBGE, 2017).

Segundo Madail et al. (2011), há uma demanda crescente por parte dos consumidores por uma alimentação mais saudável, livres de resíduos químicos, comuns no processo de produção convencional. Neste sentido, o consumidor está cada vez mais exigente quanto a obtenção de produtos com certificação de origem, qualidade, regularidade de preço e oferta, sendo este um caminho natural para que os produtores adotem essas exigências, adequando-se as maneiras mais sustentáveis de produção.

2.3 Biofertilizantes

Os biofertilizantes são regulamentados pela Instrução Normativa 46 do Ministério da Agricultura, Pecuária e Abastecimento, de 6 de outubro de 2011, sendo definidos como:

> Produtos que contém componentes ativos ou agentes biológicos, capazes de atuar, direta ou indiretamente, sobre o todo ou parte das plantas cultivadas, melhorando o desempenho do sistema de produção, sendo também isentos de substâncias proibidas pela regulamentação de orgânicos.

Pesquisas que envolvem a aplicação de substâncias naturais ou agentes biológicos, que propiciam benefícios em plantas, apresentam na literatura duas nomenclaturas: biofertilizantes e bioestimulantes. Nesta pesquisa aborda-se os

conceitos de biofertilizantes e bioestimulantes, adotando o termo biofertilizante na introdução e revisão bibliográfica. Na redação dos demais capítulos (artigos) foi utilizado o termo bioestimulante, uma vez que esta nomenclatura é melhor compreendida internacionalmente, devido a tradução, sobre o tema dos biofertilizantes.

Segundo Du Jardin (2015), um biofertilizante é qualquer inoculante bacteriano ou fúngico aplicado às plantas com o objetivo de aumentar a disponibilidade de nutrientes e sua utilização pelas plantas, independentemente do teor nutricional do próprio inoculante. Além disso, os biofertilizantes também podem ser utilizados como bioestimulantes microbianos, melhorando a eficiência nutricional das plantas. Segundo Abdel-Raouf et al. (2012), os biofertilizantes são produtos contendo microrganismos vivos ou substâncias naturais que são capazes de melhorar a qualidade química e biológica do solo, estimulando o crescimento das plantas. Já os bioestimulantes são descritos por Du jardin (2015) como substâncias, microrganismos ou a mistura de ambos, que aplicado em plantas tem o objetivo de melhorar a eficiência nutricional, a tolerância ao estresse abiótico e/ou melhorar a qualidade das culturas, independentemente do seu teor de nutrientes.

Yakhin et al. (2017) apresentaram uma perspectiva global sobre o tema de bioestimulantes. Neste estudo, os autores comentam que o termo bioestimulante ainda apresenta uma definição pobre, sendo necessário estudos na área, juntamente com órgãos jurídicos (regulamentadores), para uma melhor definição. Além disso, os autores propuseram a seguinte definição para os bioestimulantes: “produto de origem biológica com propriedades inovadoras que proporcionam aumento de produtividade, sem que sejam constituídos de nutrientes essenciais, reguladores de crescimento e complexos protetores de plantas”.

Diante disso, biofertilizantes ou bioestimulantes, oriundo de fontes naturais e que proporcione resultados positivos quando aplicado em plantas, tornam-se tecnologias interessantes a serem empregadas na agricultura, especialmente em sistemas de cultivos alternativos, como o orgânico.

Na literatura encontram-se descritas diversas fontes de biofertilizantes com potencial na aplicação na agricultura. Os L-aminoácidos, hidrolisados proteicos, extratos de macroalgas, substâncias húmicas e microalgas, são exemplos de fontes biofertilizantes a serem empregadas nos vegetais (DU JARDIN, 2015).

Pesquisas utilizando L-ácido glutâmico e hidrolisado proteico mostram em solanáceas promoção no crescimento das plantas, com impactos positivos na

produtividade e aumento na concentração de biomoléculas como clorofilas e enzimas relacionadas ao ciclo do N (COLLA et al., 2014; RÖDER et al., 2018). Além disso, os extratos de macroalgas são umas das fontes mais antigas a serem empregadas na agricultura, muito embora os efeitos da aplicação em plantas tenham sido identificados recentemente (DU JARDIN, 2015). No cultivo de cebola, Dogra e Mandradia (2012), observaram a promoção do crescimento da parte aérea das plantas e o dobro de produtividade aplicando 2.5 g/m² do extrato da macroalga *Ascophyllum nodosum*.

As substancias húmicas, por sua vez, apresentam diversos benefícios quando aplicado em plantas sendo seu efeito relatado na literatura (CANELLAS et al., 2015), já a aplicação das microalgas é recente, sendo umas das fontes de biofertilizantes pouco explorada (RONGA et al., 2019).

2.3.1 Potencial das microalgas na agricultura

Estima-se que exista no mundo em torno de 800 mil espécies de microalgas, sendo que deste total, aproximadamente 6,25% são descritas (SUGANYA et al., 2016). Esses microrganismos são capazes de produzir biomassa com potencialidade no uso em diversos setores produtivos, tais como, biocombustíveis, farmacêutico, nutrição animal e aplicação na agricultura (ISHAQ et al. 2016). Algumas espécies de microalga apresentam de 25 a 75% do seu peso seco composto de lipídeos (MALCATA, 2011), sendo possível a extração dessa biomolécula da biomassa de microalgas para a produção de biocombustível (MUBARAK et al., 2015). Além dos lipídeos, outra biomolécula de grande interesse para indústria alimentícia e de nutrição animal encontrada na biomassa das microalgas são as proteínas, pois, algumas espécies podem apresentar de 50 a 56% do seu peso seco em proteínas (ISHAQ et al., 2016).

A utilização de microalgas na agricultura é recente, sendo umas das fontes de biofertilizante pouco explorada (RONGA et al., 2019). Atualmente, pesquisas mostram resultados promissores na utilização desses microrganismos em plantas, sendo a aplicação capaz de promover o aumento das massas das plantas, ganhos na produtividade e no aumento da concentração de biomoléculas, tais como carboidratos e proteínas (DINESHKUMAR et al., 2019).

Coppens et al. (2016) realizaram pesquisa com tomate cultivado em meio hidropônico testando a biomassa de *Nannochloropsis* como biofertilizante, constatando aumento na qualidade de frutos de tomate, bem como aumento nos

níveis de açúcares e carotenoides dos frutos. Já Barone et al. (2018), observaram incrementos no sistema radicular e aumento na expressão de genes relacionados a aquisição de nutrientes pelas raízes de plantas de beterraba tratadas com as microalgas do gênero *Scenedesmus* e *Clorella*.

A aplicação foliar de microalgas em plantas, mostra-se uma técnica promissora a ser empregada. Mógor et al. (2018), aplicando a biomassa hidrolisada de *Arthrospira platensis,* observaram em mudas e em plantas a campo, conduzido em sistema orgânico, incrementos nas variáveis biométricas, bem como na concentração de poliaminas em tecido vegetal, substância esta responsável pelo aumento na divisão celular. Plaza et al. (2018), em pesquisa com biofertilizante a base de microalgas (*Scenedesmus* spp. e *Arthrospira* spp.) hidrolisadas, observaram concentrações de auxinas e citocininas presentes nas soluções. Os autores verificaram também que a aplicação foliar das soluções hidrolisadas, promoveram aumento nas massas secas da parte aérea e radicular, com aumento no número de flores e na concentração de macronutrientes em tecido vegetal.

2.3.2 Microalga *Scenedesmus subspicatus*

Da classe das Chlorophyceae, ordem Sphaeropleales e da família Scenedesmaceae, as microalgas do gênero *Scenedesmus*, segundo Bicudo e Menezes (2006), são as mais abundantes encontradas nos ambientes aquáticos e apresentam uma vasta variação morfológica dentro de cada espécie. A microalga *Scenedesmus subspicatus* é uma espécie encontrada em água doce apresentando uma ampla distribuição geográfica sendo identificada desde a Europa Ocidental e Oriental, Ásia, América do Norte e do Sul (GUIRY; GUIRY, 2019).

Pesquisas com a microalga *S. subspicatus* revelam grande potencial no uso de fitorremediação de águas poluídas por metais pesados, mostrando a habilidade na absorção de bário e cobre (KNAUER et al. 1997; THEEGALA et al. 2001). Além disso, esta cepa de microalga apresenta capacidade em se desenvolver em águas contaminadas (efluentes), sendo seu crescimento potencializado com o uso de dióxido de carbono adicionado no meio de cultivo em fotobiorreatores (GRESSLER et al. 2014). Estudos recentes com microalgas tem sido voltado a produção de combustíveis alternativos como o biodiesel, sendo esta tecnologia inovadora e ecologicamente correta. A microalga *S. subspicatus* apresenta potencial de crescimento em águas residuais, não competindo por recursos escassos como a água doce, apresentando

em sua biomassa uma concentração aproximada de 22% de lipídeos, molécula fundamental para produção de biodiesel (SUN et al. 2018).

Segundo Ishaq et al. (2016), microalga do gênero *Scenedesmus* sp. são fontes ricas de compostos bioativos que podem ser explorados em diversos ramos da indústria, como farmacêutica, cosmética, nutrição humana e também animal. Os principais componentes bioquímicos encontrados na biomassa de *Scenedesmus* sp. são proteínas (34,4%), lipídeos (21,6%) e carboidratos (20,4%) (KIRPENKO et al. 2015). Além disso, foi identificado na biomassa de microalgas do gênero *Scenedesmus* sp. o fitohormônio citocinina (ORDOG et al. 2004). Isto demonstra também o potencial das microalgas deste gênero na agricultura, pois o efeito da aplicação da biomassa de microalgas contendo citocininas promove em plantas crescimento e desenvolvimento vegetal (PLAZA et al. 2018).

2.3.2 Substâncias húmicas como biofertilizantes

As substâncias húmicas são moléculas oriundas do processo das transformações químicas e biológicas de resíduos vegetais e animais, sendo considerada a porção estável da matéria orgânica do solo e são compostas por três frações com características moleculares distintas: o ácido fúlvico, ácido húmico e humina (PRIMO et al., 2011).

A Sociedade Internacional das Substâncias Húmicas (IHSS) aborda a definição dessas moléculas como compostos ou misturas complexas e heterogêneas de materiais polidispersos formados no solos, sedimentos e águas naturais, por meio de reações químicas e bioquímicas durante a decomposição e transformação de resíduos vegetais e microbianos, através de um processo conhecido como humificação.

O ácido húmico e fúlvico, são frações das substâncias húmicas que apresentam interações positivas quando aplicados em plantas, sendo a mudança na arquitetura radicular em vegetais o resultado mais relatado (GARCIA et al., 2019). Barone et al. (2019) demonstrou em sua pesquisa com a aplicação ácido húmico extraído do mineral leonardita no cultivo de beterraba açucareira, incrementos significativos o sistema radicular. Além disso, identificou também a interação de genes relacionados a respostas hormonais em raízes que apresentaram sua expressão aumentada quando submetidas a aplicação do ácido húmico.

Canellas et al. (2009) aplicando substância húmica em plantas de milho, observaram um aumento na atividade da enzima H^+ATPase, efeito semelhante ao fitohormônio auxina. Esta enzima atua no metabolismo celular das raízes das plantas, acidificando a parede celular, ocasionando aporte de solutos para dentro da célula e promovendo o alongamento celular (CANELLAS; OLIVARES, 2014).

Além disso, a aplicação dessa fonte de biofertilizante apresenta benefícios para os cultivos agrícolas, como por exemplo, aumento no crescimento vegetal, aumento na qualidade de frutos, redução ao estresse hídrico, redução de doenças e melhora no aspecto da pós-colheita de produtos vegetais (CANELLAS, et al., 2015).

A utilização de substâncias húmicas no aumento produtivo de olerícolas se apresenta como uma prática válida. Bettoni et al. (2016) aplicando substância húmica no cultivo de cebola observaram aumento nas massas e incrementos na concentração de aminoácidos, açúcares e proteínas em bulbos. Hussein et al. (2015) por sua vez, aplicando ácido húmico e fúlvico no cultivo de tomate, observaram aumento da produtividade e qualidade de frutos, com aumento dos teores de sólidos totais e vitamina C.

Deste modo, a utilização de biofertilizantes a base de microalgas, bem como de substâncias húmicas mostram resultados promissores quando aplicados as plantas. Contudo, há poucos estudos que indiquem a interação de substâncias húmicas, tais como o ácido húmico ou fúlvico com microalgas. Neste sentido, o objetivo desta pesquisa foi avaliar as interações da aplicação de ácido húmico, ácido fúlvico e da microalga *Scenedesmus subspicatus* em plantas, no cultivo de cebola orgânica.

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3. CAPÍTULO I - MICROALGAE ASSOCIATED TO HUMIC ACID AS A NOVEL BIOSTIMULANT IMPROVING ONION GROWTH AND YIELD – ARTIGO PUBLICADO EM SCIENTIA HORTICULTURAE

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Microalgae associated to humic acid as a novel biostimulant improving onion growth and yield

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ABSTRACT

The search for new natural sources for plant growth promotion and yield gains can contribute to ensuring safe and sustainable vegetable production. The potential of microalgae as a plant biostimulant source has been investigated in recent years, while the use of humic substances is well established; however the study of the combination of both is novel, and is the aim of this work. A step-by-step approach was adopted, conducting three experiments using the microalgae *Scenedesmus subspicatus* combined with humic acid: i) rooting bioassays in a growth chamber to identify bioactivity of microalgae, humic acid and their mixtures using *Vigna radiata* as a model plant. ii) immersion treatments of two onion cultivar seedlings at transplantation which were then grown in pots in a greenhouse, iii) immersion treatments of two onion cultivar (open-pollinated and hybrid) seedlings at transplantation which were then grown at field in organic system. Growth and biochemical variables, yield and nutrient content of onion leaves and bulbs were evaluated. The bioactivity of microalgae biomass, of humic acid and their mixtures were identified on bioassays, and synergy was found. The immersion of onion seedlings in microalgae plus humic acid solutions showed growth promoting effects at the early stages, improved bulb caliber and yield of the hybrid cultivar, and incremented sugars and proteins content in bulbs. The yield gain was not related to nutrients uptake stimuli.

Keywords: *Allium cepa* L., *Scenedesmus subspicatus*, *Vigna radiata*, organic system, nutrient content.

1. INTRODUCTION

As a sustainable tool, the use of plant biostimulants is growing in interest (DU JARDIN, 2015), because it could contribute to improving yield in sustainable agriculture. Besides the usually reported biostimulant sources as kelp extracts (SZCZEPANEK et al. 2017a), humic acid (CANELLAS et al. 2015) and amino acids (RÖDER et al, 2018), the use of microalgae has recently shown positive effects for plant growth and yield gains (BATTACHARYYA et al. 2015, MÓGOR et al. 2018).

The use of microalgae *Acutodesmus dimorphus* on tomato plants (GARCIA-GONZALEZ AND SOMMERFELD, 2016) and the cyanobacteria *Arthrospira platensis* on lettuce (Mógor et al. 2017), has also shown a plant growth promoting effect. Similarly, the use of microalgae *Scenedesmus quadricauda* in sugar beet (BARONE et al. 2017) and *Scenedesmus almeriensis* in petunia plants (PLAZA et al. 2018), respectively promoted root and leaf growth.

The plant growth promotion effect of some microalgae species is related to a range of bioactive compounds released by these organisms, such polysaccharides, lipids and amino acids such as those found in *Scenedesmus* sp., with potential for use in diets for humans, animals and also in the pharmaceutical, biofuel and agriculture industries (ISHAQ et al. 2016; RENUKA et al. 2018).

Among the biostimulant sources, the humic substances, especially humic and fulvic acids are recognized for their potential to increase plant growth and development (CANELLAS et al. 2015). The most commonly reported effect of the application of humic acid on plants is that of stimulating root growth (SILVA et al. 2011) as a consequence of triggering greater expression of the H + ATPase enzyme, in a similar way to the auxin hormone (CANELLAS et al. 2009). Linked to this, the successful use of humic substances on organically grown onion seedlings was related to the improvement in root growth (BETTONI et al. 2016a).

Onion (*Allium cepa* L.) is a very important commercial crop in south region of Brazil. Currently, the region accounts for 54% of the total produced in the country of 1,6 million tons. Therefore, developing a simple, nature-friendly and efficient technique for more sustainable onion production gains in importance.

The use of microalgae and humic substances in plants has been reported, but the potential synergy of the combination of both is still unexplored. In this sense, the aim of this study was to evaluate the use of microalgae *Scenedesmus subspicatus* combined with humic acid adopting a step-by-step approach, three experiments being

conducted: i) rooting bioassays in a growth chamber to identify bioactivity of microalgae, humic acid and their mixtures using *Vigna radiata* as a model plant; ii) immersion treatments of two onion cultivar seedlings at transplantation which were then grown in pots at greenhouse, iii) immersion treatments of two onion cultivar seedlings at transplantation which were then grown at field in organic system.

2. MATERIAL AND METHODS

To obtain the biomass of the microalgae *Scenedesmus subspicatus* Chodat (synonym: *Desmodesmus subspicatus*) (Sc), the strain was provided by the "Elizabeth Aidar" Microalgae Collection from the Fluminense Federal University, Niteroi, Rio de Janeiro - Brazil. The autotrophic axenic cultivation was performed in a semi-continuous cultivation system in a photobioreactor using WC culture medium maintained at constant temperature (20-22ºC) and light (5500 lux), at the Crop Sciences Department of the Federal University of Paraná, Paraná State. After 25-day cultivation, the biomass was separated from the culture medium by centrifugation, attaining 0.95 g L−1 DW and was lyophilized, thus being ready to use.

The commercial formulation of humic acid (HA) used contained 33% organic carbon obtained by extraction, grinding, precipitation and filtration of the mineral Leonardite (Powhumus® Humintech GmbH - Germany), presenting a concentration of 8% potassium oxide (K_2O). Adopting a step-by-step approach (MÓGOR et al. 2017), three experiments were conducted.

2.1 Bioassays

According to the methodology proposed by Tripepi and George (1991) using *Vigna radiata* L. model plant, two bioassays were conducted in a growth chamber at 20ºC with 12 h illumination (photon flux intensity: 0.52–0.56 mmol/m2/sec) for 15 days, using a completely randomized design (n=4) with 8 plants per replicate. The aim was to verify the possible bioactivity of Sc, HA and synergy of their association by promoting *V. radiata* root growth.

Lyphilized microalgae *Scenedesmus subspicatus* biomass in aqueous suspensions was tested with the following treatments: (1) control with distilled water and (2) 0.15; (3) 0.30; (4) 0.45; (5) 0.60; (6) 0.75; (7) 1.00 $g.L^{-1}$ of Sc. Hereafter, the bioactivity of HA and the combinations with Sc were tested.

To identify any effect of the potassium present in the HA formula, as a positive control, treatments with KOH (potassium hydroxide) solutions corresponding to the HA potassium concentrations were added. The treatments were: (1) control with distilled water and (2) 0.0402 $g.L^{-1}$ KOH corresponding to the concentration of 0.30 $g.L^{-1}$ HA; (3) 0.0604 $g.L^{-1}$ KOH corresponding to the concentration of 0.45 $g.L^{-1}$ HA; (4) 0.1208 $g.L^{-1}$ KOH corresponding to the concentration of 0.60 $g.L^{-1}$ HA; (5) 0.30 $g.L^{-1}$ HA; (6) 0.45 $g.L^{-1}$ HA; (7) 0.60 $g.L^{-1}$ HA, and associations: (8) 0.30 $g.L^{-1}$ HA + 0.30 $g.L^{-1}$ Sc; (9) 0.45 $g.L^{-1}$ HA + 0.45$g.L^{-1}$ Sc and (10) 0.60 $g.L^{-1}$ HA + 0.60 $g.L^{-1}$ Sc.

The total root length (sum of the length of all roots) and average diameter (sum of root length separated by diameters) were determined using WinRhizo Pro® software (Regent Instr.® Canada), coupled to Epson® dual-lens scanner (V700 PHOTO model).

Data were tested using the Bartllet test and ANOVA. The Scott-knott mean test ($p< 0.01$) was applied, processed by Assistat 7.7 beta software.

2.2 Pots experiment

As the second step, based on the results of the bioassays, in May 2017 the onion 'BR-29' (open-pollinated cultivar) and 'Perfecta F1' (hybrid) (Topseed®), both commonly used by growers in South of Brazil, were sown in a nursery type seedbed under a polyethylene tunnel. 45 days after sowing (DAS) the seedlings were collected and used for pot and field experiments, which were conducted simultaneously. The seedlings presented 5 leaves, average pseudostem diameter of 3.08 mm (BR-29) and 4.32 mm (Perfecta F1) and properly developed roots.

The onion seedlings were immersed in solutions containing: (i) 0.3 $g.L^{-1}$ HA and 0.3 $g.L^{-1}$ Sc; (ii) 0.6 gL^{-1} HA + 0.6 gL^{-1} Sc and a (iii) control with distilled water for one minute. The experiment was conducted in a greenhouse using 3 L pots filled with soil, which chemical analysis indicated adequate for onion growing, as follows: 5.84 pH (H_2O), 26.31 $g.dm^{-3}$ organic matter; 49 $mg.dm^{-3}$ P; 1.32 $cmolc.dm^{-3}$ K; 5.28 $cmolc.dm^{-3}$ Ca; 3.05 $cmolc.dm^{-3}$ Mg; 0 $cmolc.dm^{-3}$ Al; 2.93 $cmolc.dm^{-3}$ Al+H; 12.58 $cmolc.dm^{-3}$ CEC, 76.7% base saturation, and 60% of clay content.

To achieve the effect of treatments on the initial biomass accumulation, at 60 days after transplanting both cultivars were collected for measurement of fresh and dry mass of leaves, fresh and dry mass of pseudostems, fresh and dry mass of roots and total chlorophyll in leaves according to Lichtenthaler (1987).

The experimental design was completely randomized in a factorial scheme with four replications (n= 4). A: cultivar, and B: i, ii and iii treatments. Each replicate comprised two pots containing three plants per pot. Data were tested by the Bartllet test and ANOVA. The Scott-knott mean test ($p< 0.01$) was applied, processed by Assistat 7.7 beta software.

2.3 Field experiment

The experiment was conducted in the organic vegetables production research area, where an organic system was implemented 13 years ago, at the Federal University of Paraná, at the geographical coordinates 25° 25 S and 49° 06′W at an altitude of 920m. The climate, according to the Köppen classification, is temperate type Cfb. Chemical analysis of the 0–20 cm layer of soil in the field indicated the average values: 6.30 pH (H2O), 33.30 g.dm^{-3} organic matter; 133.10 mg.dm^{-3} P; 1.44 cmolc.dm^{-3} K; 9.30 cmolc.dm^{-3} Ca; 4.30cmolc.dm^{-3} Mg; 0 cmolc.dm^{-3} Al; 3.7 cmolc.dm-3 Al+H; 18.34 cmolc.dm-3 CEC and 80% base saturation. Seven days prior to transplanting, the soil was prepared with the incorporation of 8 t ha^{-1} organic compost with the following average values: C = 30.3 g kg^{-1}; N = 30.3 g kg^{-1}; P = 8.5 g kg^{-1}; K = 6.6 g kg^{-1}; Ca = 8.1 g kg^{-1}; Mg = 4.1 g kg^{-1}. The soil fertilization was done according to the Brazilian regulation for organic agriculture.

The onion seedlings were transplanted in beds with a dimension of 1.20 x 24 m, with spacing of 30 cm between rows and 10 cm between plants, distributed in 4 planting rows, equivalent to a plant population of 230.000 per hectare. The treatments and the method of application were the same as described above in the pot experiment, being distributed in 1.20 x 1.0-m plots with a completely randomized design (n= 4) and factorial scheme.

2.3.1 Biometric and yield analysis

At 150 days after transplanting, about 80% of the plants showed pseudostem collapse indicating the proper harvest time. The plants were harvested and kept on plots over the soil for a week for full leaf senescence. Eight bulbs were collected per plot for fresh and dry mass determination using a digital scale, and the ratio between fresh and dry mass was calculated. Another twelve bulbs per plot were collected for bulb classification according their diameters (caliber) as follows: caliber 2 (35 to 50

mm), caliber 3 (50 to 70 mm) and caliber 4 (70 to 90 mm) according Brazilian market bulbs classification, and the total and commercial (caliber 3 to 5) yield was determined.

2.3.2 Chemical analysis

Four onion plants per plot were randomly collected to quantify macro and micronutrient contents (K, P, Ca, Mg, Cu, Mn, Fe, Zn and B) of bulbs and leaves. After drying in a forced air circulation oven (65°C ± 50°C), samples with 0.3 g of dry mass were diluted in HNO3 and dissolved in H_2O_2, subsequently read by means of Perkin Elmer Optima 4300 Induction Plasma Optical Emission Spectroscopy (Perkin Elmer®, USA) in triplicate. The quantification of nitrogen (N-total) was carried out by combustion in the CHONS analyzer (model Vario EL III).

2.3.3 Biochemical analysis

For the determination of the total and reducing sugars in bulbs, the methodology described by Maldonade et al. (2013) was used. The free amino acids in bulbs were extracted following Winters et al. (2002) and the colorimetric reaction was performed according to Magné and Larher (1992). Soluble proteins were determined using the methodology described by Bradford (1976).

Data of biometric, chemical and biochemical analysis were tested using the Bartllet test and ANOVA. The Scott-knott mean test ($p< 0.01$) was applied. Data of bulb caliber were submitted to the non-parametric Kruskal-Wallis test ($p<0.05$), all processed by Assistat 7.7 beta software.

3. RESULTS

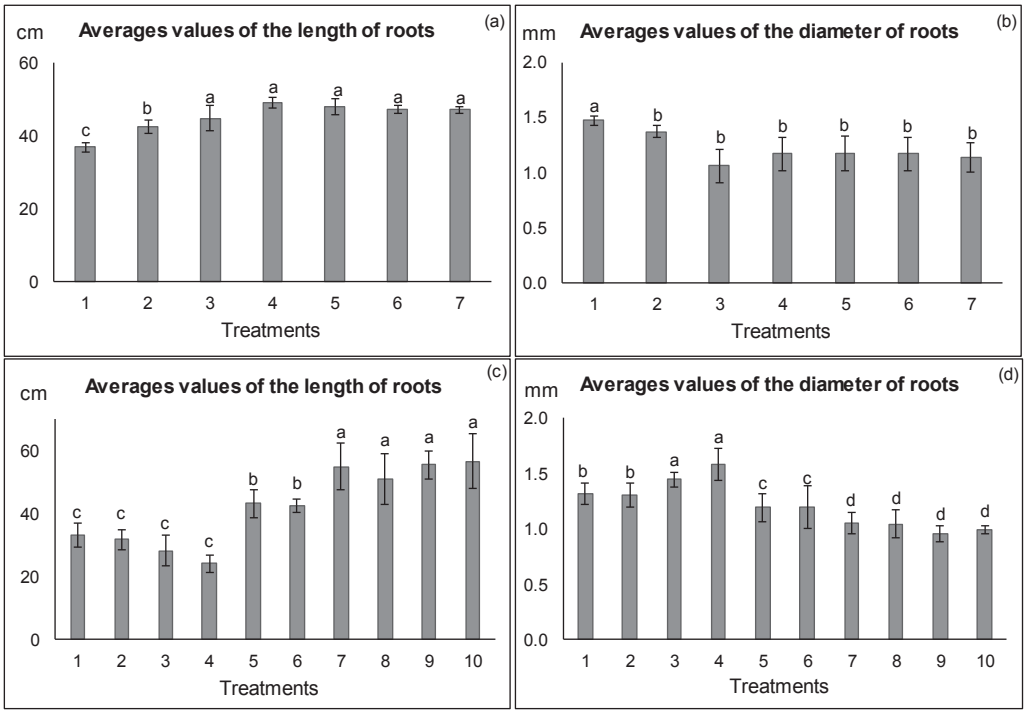
3.1 Bioassays

The *V. radiata* total root length and average diameter data showed a growth promoting effect of Sc more pronounced from 0.30 $g.L^{-1}$, without deferring at increasing concentrations (Fig. 1a). The increase in length (sum of the length of all roots) implied in reduction of root diameter (sum of root length separated by diameters) (Fig. 1b).

The results also indicated that the KOH in commercial formula did not act to promote root growth of *V. radiata* (Fig. 1 c - treatments 2 to 4), allowing the

characterization of HA bioactive effect, being part of the organic fraction (33% of organic carbon) in the formula (Fig. 1 c - treatments 5 to 7).

FIGURE 1 - ROOT LENGTH AND DIAMETER OF *Vigna radiata* PLANTS UNDER TREATMENTS WITH MICROALGAE *Scenedesmus subspicatus* LYOPHILIZED BIOMASS AQUEOUS SOLUTIONS (A AND B), AND WITH SOLUTIONS CONTAINING KOH, HUMIC ACID ALONE, AND MIXINGS OF MICROALGAE PLUS HUMIC ACID (C AND D). TREATMENTS AT (A) AND (B): (1) CONTROL, (2) *Scenedesmus subspicatus* (Sc) 0.15 g.L^{-1}; (3) 0.30 g.L^{-1}; (4) 0.45 g.L^{-1}; (5) 0.60 g.L^{-1}; (6) 0.75 g.L^{-1}; (7) 1.00 g.L^{-1}. TREATMENTS AT (C) AND (D): (1) CONTROL; (2) 0.0402 g.L^{-1} KOH CORRESPONDING TO A CONCENTRATION OF 0.30 g.L^{-1} HA; (3) 0.0604 g.L^{-1} KOH CORRESPONDING TO THE CONCENTRATION OF 0.45 g.L^{-1} HA; (4) 0.1208 g.L^{-1} KOH CORRESPONDING TO THE CONCENTRATION OF 0.60 g.L^{-1} HA; (5) 0.30 g.L^{-1} HA; (6) 0.45 g.L^{-1} HA; (7) 0.60 g.L^{-1} HA; (8) 0.30 g.L^{-1} HA + 0.30 g.L^{-1} Sc; (9) 0.45 g.L^{-1} HA + 0.45 g.L^{-1} Sc AND (10) 0.60 g.L^{-1} HA + 0.60 g.L^{-1} Sc. COLUMNS WITH THE SAME LETTER DO NOT DIFFER STATISTICALLY ($P < 0.01$) ACCORDING TO SCOTT-KNOTT TEST. BARS REPRESENT STANDARD ERROR



The root length improvements achieved around 23% at concentrations of 0.30 g.L^{-1} HA and 0.45 g.L^{-1} HA (Fig. 1 c - treatments 5 and 6) compared to the control. The highest concentration of HA (treatment 7) and the mixings with Sc (treatments 8 to 10) did not differ, showing average of root length increments of 39% when compared to the control (Fig. 1 c). These results suggest synergy of HA+Sc mixes at lowest concentrations (Treatments 8 and 9) up to the concentration of HA of 0.60 g.L^{-1}.

As observed in the Sc alone bioassay (Fig. 2 a b), the length improvement also implied in reduction of root diameter, notably for treatments 7 to 10, around 29% when compared to the control (Fig. 1d).

3.2 Pot experiment

Aiming for a simple technique (seedling immersion at transplantation) to improve onion yield in a nature friendly way, the mixed solutions with the lower (0.30 $g.L^{-1}$ Sc + 0.30 $g.L^{-1}$ HA) and the higher (0.60 $g.L^{-1}$ Sc + 0.60 $g.L^{-1}$ HA) concentrations of Sc and HA were tested by immersing onion seedlings of cultivars BR-29 and Perfecta F1 for one minute before transplantation. The treatments with 0.30 $g.L^{-1}$ Sc + 0.30 $g.L^{-1}$ HA (3SH) and 0.60 $g.L^{-1}$ Sc + 0.60 $g.L^{-1}$ HA (6SH) upon 60 days after transplanting showed promotion effect on plant growth (Table 1).

Comparing the cultivars, the 'Perfecta F1' ('P') presented a higher fresh mass of leaves, pseudostems and roots, and also dry mass of pseudostems (Table 1 a, c, e, d - respectively) than BR-29 ('B'). There was no difference in dry mass of leaves and roots (Table 1 b, f). These results indicate that the hybrid onion ('P') accumulated more photoassimilates in pseudostems regarding improvement in dry mass.

Comparing the cultivars at immersion treatments, there were almost no differences between 3SH and 6SH, both incrementing biomass accumulation of onion plants compared to the control, though just 'P' showed improvements in pseudostems fresh mass (Table 1c).

TABLE 1 - VALUES OF BIOMETRIC VARIABLES OF ONION CULTIVARS AT 60 DAYS AFTER TRANSPLANTING TO POTS IN THE GREENHOUSE, WHERE SEEDLINGS WERE IMMERSED IN SOLUTIONS WITH MIXES OF THE MICROALGAE *Scenedesmus subspicatus* BIOMASS (Sc) AND HUMIC ACID (HA). DATA WERE OBTAINED USING SIX WHOLE ONION PLANTS BY REPLICATION. EACH REPLICATE COMPRISED TWO POTS CONTAINING THREE PLANTS PER POT

(a) Fresh mass of leaves (g)					**(b) Dry mass of leaves (g)**				
	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$		0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	9.8 ± 1.65	12 ± 1.71	11.5 ± 1.70	11.1 b	B	0.58 ± 0.10	0.73 ± 0.11	0.69 ± 0.06	0.67 a
P	11.3 ± 2.78	14.4 ± 2.68	15.9 ± 1.74	13.9 a	P	0.57 ± 0.13	0.80 ± 0.12	0.93 ± 0.10	0.77 a
$\bar{X}$	10.58 b	13.20 a	13.72 a		$\bar{X}$	0.57 b	0.76 a	0.81 a	
C	**				C	ns			
T	*				T	*			
C x T	ns				C x T	ns			
(c) Fresh mass of the pseudostem (g)					**(d) Dry mass of the pseudostem (g)**				
	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$		0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	3.56 ± 0.78 bA	4.01 ± 0.41 bA	4.01 ± 0.37 bA	3,863	B	0.258 ± 0.07	0.294 ± 0.05	0.293 ± 0.06	0.282 b
P	4.77 ± 0.99 aB	6.45 ± 0.49 aA	7.18 ± 0.97 aA	6,137	P	0.274 ± 0.05	0.444 ± 0.07	0.54 ± 0.17	0.419 a
$\bar{X}$	4.17	5,233	5,597		$\bar{X}$	0.266 b	0.393 a	0.417 a	
C	**				C	**			
T	**				T	*			
C x T	*				C x T	ns			
(e) Fresh mass of roots (g)					**(f) Dry mass of roots (g)**				
	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$		0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	7.47 ± 1.76	8.22 ± 1.95	8.54 ± 0.83	8.07 b	B	0.261 ± 0.03	0.301 ± 0.08	0.289 ± 0.04	0.283 a
P	7.56 ± 1.14	10.04 ± 1.11	10.61 ± 0.66	9.40 a	P	0.218 ± 0.05	0.323 ± 0.04	0.351 ± 0.03	0.297 a
$\bar{X}$	7.51 b	9.13 a	9.57 a		$\bar{X}$	0.239 b	0.312 a	0.320 a	
C	*				C	ns			
T	*				T	*			
C x T	ns				C x T	ns			

Onion cultivars: 'B'= BR-29. 'P'= Perfecta-F1. Immersion treatments: 0,0 g.L^{-1} (control), 0.3 g.L^{-1} (0.30 g.L^{-1} Sc + 0.30 g.L^{-1} HA), 0.6 g.L^{-1} (0.60 g.L^{-1} Sc + 0.60 g.L^{-1} HA). Means followed by the same capital letter at line and lowercase at column at factorial interaction (c), and lower case letters at means ($\bar{X}$) are not different according to Scott-Knott test at 5% probability ($p < 0.05$) (n=4) +/-SD. ANOVA: ns = not significant, * and ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. C = cultivars, T = treatments and C x T = interactions.

3.3 Field experiment

Simultaneously with the pot experiment, seedlings immersed in 3SH and 6SH were organically grown. Data showed factorial interaction among cultivars and immersion treatments for fresh and dry masses of bulbs and fresh/dry mass ratio (Table 2).

Comparing cultivars, 'P' bulbs presented higher fresh and dry masses than 'B'. Comparing immersion treatments, both 3SH and 6SH improved 'P' bulbs fresh and dry masses, yet did not alter 'B'.

TABLE 2 - VALUES OF FRESH MASS, DRY MASS AND FRESH/DRY MASS RATIO OF ORGANICALLY GROWN ONION (PERFECTA F1 AND BR-29 CULTIVARS) BULBS WHOSE SEEDLINGS WERE IMMERSED ON SOLUTIONS WITH MIXES OF THE MICROALGAE *Scenedesmus subspicatus* BIOMASS (Sc) AND HUMIC ACID (HA).

(a) Fresh mass of bulbs (g)

	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	389.9 ± 88.16 bA	438.1 ± 56.74 bA	404.4 ± 76.28 bA	410.8 b
P	1250.4 ± 109.23 aB	1608.7 ± 97.31 aA	1537.5 ± 140.80 aA	1465.5 a
$\bar{X}$	820.2 b	1023.4 a	971.0 a	

C	**
T	**
C x T	**

(b) Dry mass of bulbs (g)

	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	46.85 ± 9.26 bA	49.06 ± 4.07 bA	48.42 ± 7.12 bA	48.11 b
P	80.98 ± 7.42 aB	122.30 ± 6.32 aA	113.7 ± 17.81 aA	105.69 a
$\bar{X}$	63.92 b	83.68 a	81.09 a	

C	**
T	**
C x T	**

(c) Fresh/dry mass ratio of bulbs (g)

	0.0 g.L^{-1}	0.3 g.L^{-1}	0.6 g.L^{-1}	$\bar{X}$
B	8.30 ± 0.50 bA	8.94 ± 0.61 bA	8.33 ± 0.76 bA	8.52 b
P	15.44 ± 0.39 aA	13.15 ± 0.43 aB	13.66 ± 0.47 aB	14.08 a
$\bar{X}$	11.87 a	11.04 b	10.99 b	

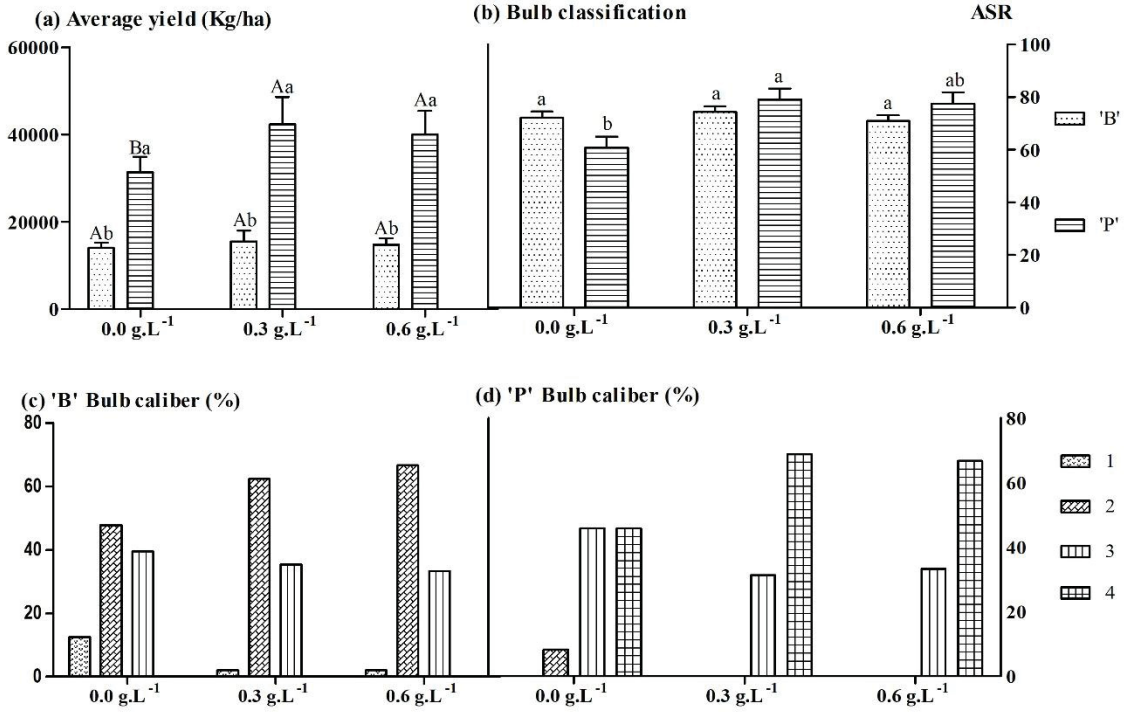
C	**
T	ns
C x T	**

Onion cultivars: 'B'= BR-29. 'P'= Perfecta-F1. Immersion treatments: 0,0 g.L^{-1} (control), 0.3 g.L^{-1} (0.30 g.L^{-1} Sc + 0.30 g.L^{-1} HA), 0.6 g.L^{-1} (0.60 g.L^{-1} Sc + 0.60 g.L^{-1} HA). Means followed by the same capital letter at line and lower case at column are not different according to Scott-Knott test at 5% probability ($p < 0.05$) (n=4) +/-SD. ANOVA: ns = not significant, * and ** = significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. C = cultivars, T = treatments and C x T = interactions.

As a consequence of biomass accumulation in bulbs, the average values of the sum of the ranks (ASR) were altered, enhancing the diameter of 'P' bulbs in SH treatments, that is, there was an increase in the number of bulbs with a larger caliber than the control (Figure 2b). The 'B' bulb population was not affected by the treatments (Figure 2b). The percentage of the bulbs per caliber indicated that 'P' concentrated at caliber 4 (70 to 90 mm) more than at 3 (50 to 70 mm) at SH treatments (Figure 2 d), while 'B' at caliber 2 (35 to 50 mm) more than at 3 (Figure 2 c).

In the same plant population, bulb caliber represents the main yield variable, as shown at figure 2(a), with 'P' presenting yield 2.5 times greater than 'B'. The SH treatments did not alter 'B' caliber and as a consequence the yield, increased 'P' yield remarkably, achieving 34% for 3SH and 27%, for 6SH.

FIGURE 2 - VALUES OF YIELD (A), SUM OF THE RANKS OF BULBS (ASR) (B) AND PERCENTAGE OF CLASSIFICATION PER CALIBER (C AND D) OF ORGANICALLY GROWN ONION CULTIVARS WHOSE SEEDLINGS WERE IMMERSED ON SOLUTIONS WITH MIXTURES OF THE MICROALGAE *Scenedesmus subspicatus* BIOMASS (Sc) AND HUMIC ACID (HA). ONION CULTIVARS: 'B'= BR-29, 'P' = PERFECTA F1. IMMERSION TREATMENTS: 0,0 G.L^{-1} (CONTROL), 0.30 G.L^{-1} (0.30 G.L^{-1} Sc + 0.30 G.L^{-1} HA) AND 0.60 G.L^{-1} (0.60 G.L^{-1} Sc + 0.60 G.L^{-1} HA). THE FIGURE (A): COLUMNS WITH THE SAME LETTER DO NOT DIFFER STATISTICALLY (P<0.01) ACCORDING TO SCOTT-KNOTT TEST (N=4); ANOVA: NS = NOT SIGNIFICANT, * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY. CAPITAL LETTERS = TREATMENTS, LOWERCASE LETTERS = CULTIVARS. THE FIGURE (A AND B): BARS REPRESENT STANDARD ERROR. THE FIGURE (B): VALUES OF THE SUM OF THE RANKS (ASR) OF ONION BULBS CALIBER ACCORDING TO KRUSKAL-WALLIS NON-PARAMETRIC TEST, WITH THE SAME LETTER ARE NOT DIFFERENT AT 5% PROBABILITY (P<0.05). THE FIGURES (C AND D): CALIBER: 1 (<35 MM), 2 (35 TO 50 MM), 3 (50 TO 70 MM) AND 4 (70 TO 90 MM).



Comparing cultivars no statistical differences were found for nutrient content in leaves, or even in bulbs (Table 3). On 'P' leaves the average values of macronutrients follow the decreasing order: N (21.79 g.kg^{-1}) > K (12.72 g.kg^{-1}) > Ca (11.35 g.kg^{-1}) > P (3.15 g.kg^{-1}) = Mg (3.17 g.kg^{-1}), and micronutrients of: Fe (2086.00 mg.kg^{-1}) > B (23.54 mg.kg^{-1}) > Mn (20.30 mg.kg^{-1}) > Zn (15.18 mg.kg^{-1}) > Cu (6.31 mg.kg^{-1}). On 'B' leaves the decreasing order was: N (20.13 g.kg^{-1}) > K (15.72 g.kg^{-1}) > Ca (7.19 g.kg^{-1}) > P (3.59g.kg^{-1}) > Mg (2.45 g.kg^{-1}), and Fe (1858.36 mg.kg^{-1}) > Zn (11.13 mg.kg^{-1}) > B (26.03 mg.kg^{-1}) > Mn (13.13 mg.kg^{-1}) > Cu (5.08 mg.kg^{-1}).

On 'P' bulbs, the average values follow the decreasing order: N (16.08 g.kg^{-1}) = K (16.11 g.kg^{-1}) > P (3.66 g.kg^{-1}) > Ca (2.85 g.kg^{-1}) > Mg (1.74 g.kg^{-1}); and Fe (914.92 mg.kg^{-1}) > B (15.33 mg.kg^{-1}) > Mn (10.58 mg.kg^{-1}) > Zn (22.02 mg.kg^{-1}) > Cu (4.55 mg.kg^{-1}). On 'B' was: N (16.95 g.kg^{-1}) > K (13.51 g.kg^{-1}) > P (4.21 g.kg^{-1}) > Ca (2.15

g.kg^{-1}) > Mg (1.46 g.kg^{-1}); and Fe (314.92 mg.kg^{-1}) > Zn (26.53 mg.kg^{-1}) > B (11.45 mg.kg^{-1}) > Mn (6.53 mg.kg^{-1}) > Cu (5.60 mg.kg^{-1}).

TABLE 3 - NUTRIENT CONTENTS IN LEAVES AND BULBS OF ORGANICALLY GROWN ONION CULTIVARS WHOSE SEEDLINGS WERE IMMERSED ON SOLUTIONS WITH MIXTURES OF THE MICROALGAE *Scenedesmus subspicatus* BIOMASS (Sc) AND HUMIC ACID (HA). ONION CULTIVARS ('B'= BR-29, 'P' = PERFECTA-F1). IMMERSION TREATMENTS: 0,0 g.L^{-1} (CONTROL), 0.30 g.L^{-1} (0.30 g.L^{-1} SC + 0.30 g.L^{-1} HA), 0.60 g.L^{-1} (0.60 g.L^{-1} SC + 0.60 g.L^{-1} HA). THE SAME LOWER CASE LETTERS (TREATMENTS) ARE NOT DIFFERENT ACCORDING TO SCOTT-KNOTT TEST AT 5% PROBABILITY (P <0.05) (N=4) +/-SD. AVERAGES WITH * ARE DIFFERENT BY THE SCOTT-KNOTT TEST AT 5% PROBABILITY (P <0.05)

Leaf

		N (mg.g^{-1})	P (mg.g^{-1})	K (mg.g^{-1})	Ca (mg.g^{-1})	Mg (mg.g^{-1})	Cu (µg.g^{-1})	Mn (µg.g^{-1})	Fe (µg.g^{-1})	Zn (µg.g^{-1})	B (µg.g^{-1})
B'	Control	19.92 ± 1.08 a	3.60 ± 0.45 a	14.92 ± 1.32 a	7.07 ± 0.84 a	2.38 ± 0.11 a	5.37 ± 0.64 a	13.13 ± 2.22 a	1286 ± 287 a	13.29 ± 2.55 a	24.55 ± 1.58 a
	3SH	20.10 ± 0.44 a	3.45 ± 0.59 a	15.52 ± 0.43 a	7.73 ± 0.79 a	2.55 ± 0.13 a	5.03 ± 0.76 a	14.39 ± 2.60 a	1000 ± 203 a	10.51 ± 1.08 a	27.39 ± 2.56 a
	6SH	20.37 ± 0.88 a	3.74 ± 0.22 a	15.93 ± 1.28 a	6.79 ± 0.50 a	2.43 ± 0.12 a	4.85 ± 0.25 a	11.88 ± 0.66 a	982 ± 78.9 a	9.60 ± 0.71 a	26.17 ± 2.18 a
Average ('B')		20.05	3.59	15.46 *	7.2	2.45	4.98	14.13	1100	11.13	26.04 *
P'	Control	21.92 ± 0.50 a	3.07 ± 0.54 a	11.8 ± 0.18 a	11.51 ± 0.57 a	3.24 ± 0.13 a	6.23 ± 0.90 a	20.44 ± 6.31 a	2083 ± 216 a	13.83 ± 1.48 a	23.63 ± 1.44 a
	3SH	21.95 ± 0.64 a	2.93 ± 0.61 a	12.29 ± 0.86 a	11.59 ± 0.69 a	3.31 ± 0.16 a	6.73 ± 0.81 a	20.46 ± 5.63 a	2319 ± 287 a	15.89 ± 3.26 a	24.69 ± 1.70 a
	6SH	21.52 ± 1.65 a	3.47 ± 0.53 a	14.08 ± 0.86 a	10.96 ± 0.12 a	2.98 ± 0.81 a	5.98 ± 0.72 a	20.00 ± 6.70 a	1858 ± 240 a	15.83 ± 3.67 a	22.30 ± 2.91 a
Average ('P')		21.80 *	3.16	12.72	10.69 *	3.18 *	6.08 *	20.30 *	2086 *	15.19 *	23.54

Bulb

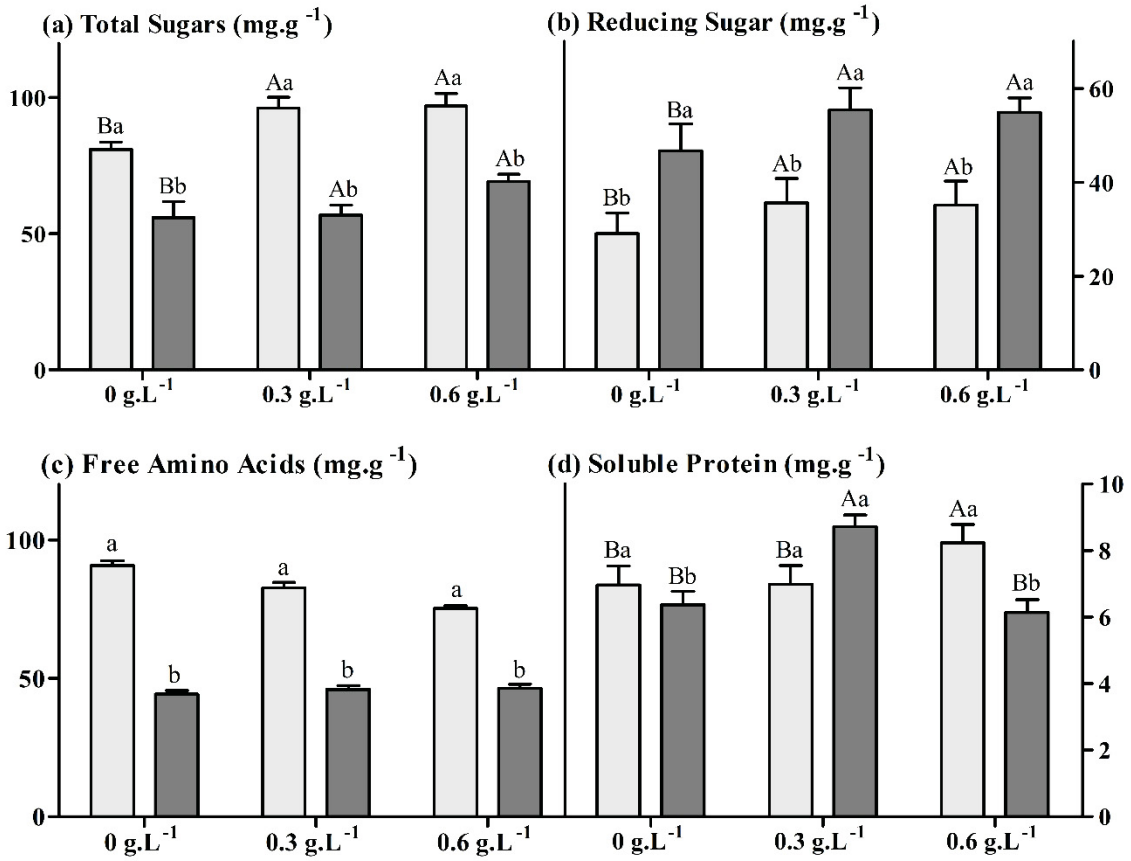
		N (mg.g^{-1})	P (mg.g^{-1})	K (mg.g^{-1})	Ca (mg.g^{-1})	Mg (mg.g^{-1})	Cu (µg.g^{-1})	Mn (µg.g^{-1})	Fe (µg.g^{-1})	Zn (µg.g^{-1})	B (µg.g^{-1})
B'	Control	14.65 ± 1.45 b	3.95 ± 0.48 a	13.00 ± 0.19 a	2.16 ± 0.16 a	1.43 ± 0.09 a	5.69 ± 0.54 a	5.75 ± 0.63 a	305 ± 19.3 a	24.02 ± 2.01 a	10.91 ± 1.36 a
	3SH	17.18 ± 2.85 a	4.20 ± 0.31 a	13.46 ± 0.65 a	2.06 ± 0.17 a	1.42 ± 0.06 a	5.49 ± 0.79 a	6.86 ± 1.07 a	333 ± 9.60 a	26.20 ± 4.51 a	11.74 ± 1.23 a
	6SH	19.02 ± 2.10 a	4.49 ± 0.32 a	14.09 ± 0.78 a	2.23 ± 0.23 a	1.54 ± 0.21 a	5.63 ± 0.70 a	7.00 ± 1.22 a	305 ± 5.90 a	29.38 ± 2.74 a	11.70 ± 2.09 a
Average ('B')		16.95	4.22 *	13.52	2.15	1.46	5.60 *	6.37	314	26.53 *	11.45
P'	Control	15.97 ± 0.30 a	3.37 ± 0.10 a	15.82 ± 0.85 a	2.87 ± 0.44 a	1.74 ± 0.11 a	4.16 ± 0.58 a	9.57 ± 1.47 a	900 ± 70.1 a	20.55 ± 2.22 a	14.15 ± 2.06 a
	3SH	16.35 ± 0.60 a	3.82 ± 0.44 a	16.43 ± 0.92 a	2.88 ± 0.25 a	1.75 ± 0.05 a	4.81 ± 0.45 a	11.38 ± 2.75 a	941 ± 105 a	22.42 ± 2.70 a	16.21 ± 2.03 a
	6SH	15.94 ± 0.38 a	3.79 ± 0.53 a	16.08 ± 0.99 a	2.82 ± 0.19 a	1.73 ± 0.08 a	4.68 ± 0.67 a	10.80 ± 1.93 a	950 ± 145 a	23.10 ± 3.79 a	15.63 ± 2.46 a
Average ('P')		16.09	3.66	16.08 *	2.86 *	1.74 *	4.55	10.59 *	930 *	22.02	15.33 *

N was the macronutrient most accumulated in leaves and bulbs in both cultivars, but was equal to K in ‘P’ bulbs. The Fe was the micronutrient most accumulated in leaves and bulbs in both cultivars. Comparing the immersion treatments, only the N content on ‘B’ bulbs was incremented by SH (Table 3). However, taking into account that SH immersion did not affect ‘B’ bulb caliber or yield (Figure 2), and that the ‘P’ nutrient contents did not differ from control, then the caliber and yield gains are not related to nutrient uptake stimuli.

On the other hand, biochemical changes were identified. In Fig. 3a the total sugars of ‘B’ presented increases by 48% when compared to ‘P’. However, the content of reducing sugars (Fig. 3b) of ‘P’ presented a higher concentration (57%) when compared to ‘B’. The treatments using SH increased total and reducing sugar contents in the onion bulbs when compared to the control.

The total free amino acids (Fig. 3c) content in ‘B’ bulbs was greater (82%) when compared to ‘P’, with no effect of treatments on both cultivars. The soluble proteins (Fig. 3d) showed interaction between treatments and cultivars. The 6SH significantly increased the soluble protein content (18%) in the bulbs of ‘B’ in relation to the other treatments. While in ‘P’, the treatment 3SH promoted an increase of 37% in the concentration of soluble proteins when compared to the control.

FIGURE 3 - CONTENTS OF TOTAL SUGARS (A), REDUCING SUGAR (B), TOTAL FREE AMINO ACIDS (C) AND SOLUBLE PROTEINS (D) ON BULBS OF ORGANICALLY GROWN ONION CULTIVARS WHOSE SEEDLINGS WERE IMMERSED FOR ONE MINUTE ON SOLUTIONS WITH MIXTURES OF THE MICROALGAE *Scenedesmus subspicatus* BIOMASS (SC) AND HUMIC ACID (HA). ONION CULTIVARS (LIGHT GRAY = BR-29, DARK GRAY = PERFECTA F1). IMMERSION TREATMENTS: 0.30 g.L^{-1} (0.30 g.L^{-1} SC + 0.30 g.L^{-1} HA), 0.60 g.L^{-1} (0.60 g.L^{-1} SC + 0.60 g.L^{-1} HA). COLUMNS WITH THE SAME LETTER DO NOT DIFFER STATISTICALLY (P< 0.01) ACCORDING TO SCOTT-KNOTT TEST (N=4). CAPITAL LETTERS = IMMERSION TREATMENTS. LOWERCASE LETTERS = CULTIVARS. BARS REPRESENT STANDARD ERROR



4. DISCUSSION

Root growth promotion by *Scenedesmus subspicatus* (*Desmodesmus subspicatus*) indicates the bioactivity of this microalgae biomass, similar to the findings for Chlorophyceae Class *S. quadricauda*, reported by Barone et al. (2017).

The Chlorophyceae Class microalgae, known as green microalgae, are usually reported as lipid sources for biofuel (ISHAQ et al. 2016). At the same time, recent reports highlight the potentialities of some Chlorophyceae being biostimulant sources for plant growth promotion, such as *Acutodesmus dimorphus* (GARCIA-GONZALEZ AND SOMMERFELD, 2016) and *Scenedesmus almeriensis* (PLAZA et al. 2018).

In general, the effect on plant growth promotion may be related to the presence of phytohormone cytokinin in microalgae strains, which is capable of inducing cell division (STIRK et al. 2013), among other possible bioactive molecules such polysaccharides, amino acids and polyamine spermine also related to cell multiplication (MÓGOR et al. 2017; RENUKA et al. 2018). On the other hand, the HA action in inducing plant root growth is well characterized by the presence of signaling molecules that induce responses as the phytohormone auxin (CANELLAS et al. 2009).

Root elongation and narrowing is an effect 'like' auxin signalization, stimulating cell elongation and root hair initiation by HA (CANELLAS et al. 2015), and besides that, related to potential bioactive molecules on microalgae (ISHAQ et al. 2016) able to promote plant growth (Barone et al. 2017), as observed on *V. radiata* bioassays (Fig. 1). This allows the identification of microalgae bioactivity on root growth promotion, and characterizes the 'auxin like' effect of commercial HA, without interference of the KOH in the formula (Fig. 1 c d).

Onion (*Allium cepa* L.) is a very important commercial crop in Southern Brazil, being the main activity of a large number of small growers, classifying it as a family crop, and making it impactful socioeconomically (BETTONI et al. 2016b).

The performance of the onion crop is highly dependent on the initial growth after transplantation, giving great importance to early stages of plants development (BETTONI et al. 2016a). 'B' and 'P' seedling immersion on 3SH and 6SH improved the plant growth until 60 days after transplanting, reflected in higher biomass accumulation, in particular in the hybrid onion pseudostems (Table 1).

The improvements in biomass accumulation could be related to increases in chlorophylls, which was reported as an effect of macroalgae foliar sprays on grown onion (SZCZEPANEK et al. 2017a) and humic acid on chrysanthemum (FAN et al. 2014). However, using SH treatments on 'P' and 'B', differences in chlorophyll were not found. The average values of total chlorophyll in leaves of 'P' and 'B', respectively were: control (0.40 and 0.46 $\mu g.g^{-1}$), 3SH (0.52 and 0.44 $\mu g.g^{-1}$) and 6SH (0.46 and 0.42 $\mu g.g^{-1}$).

Other pathways may stimulate the accumulation of biomass without necessarily changing chlorophylls, such as the reactivity of humic acid in the rhizosphere releasing auxin-like molecules, promoting increases in H + ATPase enzyme activity, resulting in higher biomass accumulation as a consequence of cell expansion (JINDO et al. 2012). The effect of a higher biomass accumulation in plants could be also attributed to the presence of molecules with hormonal action in green microalgae, such as cytokinins

and polyamines, that result in greater cell division (STIRK et al. 2013; MÓGOR et al. 2017).

The results obtained in pots (Table 1) corroborate that found in bioassays (Figure 2 c), indicating that SH solutions were absorbed stimulating plant growth, characterizing their biostimulant effect (DU JARDIN, 2015).

Simultaneously to the pot experiment, seedlings immersed in 3SH and 6SH were organically grown. Data has shown factorial interaction among cultivars and immersion treatments for fresh and dry masses of bulbs and their ratio, indicating more water accumulation by hybrid onion bulbs compared to the open-pollinated cultivar, and also higher fotoassimilates accumulation in 'P' bulbs compared to control (Table 2) enhancing their caliber (Figure 3 b).

Similarly, the use of humic acid increased fresh and dry mass of chrysanthemum (Fan et al. 2014), the use of macroalgae *Ecklonia maxima* increased root size of carrots (SZCZEPANEK et al. 2017b), and the use of microalgae increased fresh and dry mass on red beet (MÓGOR et al. 2018), thereby resulting in lower fresh/dry mass ratio compared to control, indicating increment of photoassimilates accumulation in sink organs.

Bulb caliber represents the main yield variable, as shown at figure 3(a), with 'P' presenting a yield 2.5 times greater than 'B'. These results indicate that the effect of SH immersion treatments are cultivar dependent, an effect already observed at 60 days after planting when 'P' presented highest pseudostems fresh mass (Table 1 c). The SH treatments increased 'P' yield on 34% for 3SH and 27%, for 6SH.

The biostimulant effect of humic substances were previously reported for onion yield increases (KANDIL et al. 2013), as well as in rice with microalgae increasing growth and yield (DINESHKUMAR et al. 2018). Some authors relate the biostimulat effects to improvements in nutrient acquisition by plants (BARONE et al. 2017), nevertheless, this effect was not found for SH treatments (Table 3).

Only the N content in 'B' bulbs was increased by SH (Figure 4 a). However, taking into account that SH immersion did not affect 'B' bulb caliber or yield (Figure 3), and that the 'P' nutrient contents did not differ from the control, thus the caliber and yield gains are not related to nutrient uptake stimuli. However, biochemical changes in bulbs could explain SH effects.

About 80% of onion bulb dry matter consists of non-structural carbohydrates, the main carbohydrate components being the reducing sugars glucose and fructose, as well as the non-reducing sucrose, and fructo-oligosaccharides (ZHANG et al. 2016).

The sugars are the primary products of photosynthesis, and their source-to-sink transport is determinant to plant growth, being transported in the phloem and accumulating in the sink, thus stimulating the water influx on sink as observed in Table 2, with higher dry mass and also water accumulation in ‘P’, reflecting on caliber and yield gains, since this carbohydrates in the water balance in bulbs (LEMOINE et al. 2013).

The total sugars in bulbs of both cultivars were improved by SH (Figure 3a), probably as a consequence of the improvement in the initial growth of plants (Table 1), and also suggesting that seedling immersion stimulated the source-to-sink transport during ‘P’ bulbs swelling stages.

Biostimulants could improve total soluble protein content in plants through better N assimilation and stimulation of the amino acid metabolism (NARDI et al. 2016). However, the effect of SH immersions did not show a clear action on N metabolism, even though the treatment 3SH promoted an increase of 37% in the concentration of soluble proteins in ‘P’ when compared to the control, an effect not repeated by 6SH.

Although there was no effect on chlorophylls, the biostimulant action of SH immersion was strongly related to carbon metabolism, improving sugars accumulation in onion bulbs, increasing caliber and yield in the hybrid cultivar. Further investigations could clarify the open questions that sugars accumulation was related to initial growth stimuli, to improvement in source-to-sink flow, or both, opening new possibilities for use of SH on foliar sprays or drip irrigation. It is possible to consider SH as a biostimulant, being a simple to use nature friendly new tool for sustainable onion production.

5. CONCLUSION

The bioactivity of humic acid (HA), microalgae *Scenedesmus subspicatus* (Sc) and their mixtures was identified on bioassays, and synergy at low concentrations was found. The immersion of onion seedling in solutions with 0.30 $g.L^{-1}$ Sc + 0.30 $g.L^{-1}$ HA has shown a plant growth promotion effect on onion early stages, improved bulb caliber and yield of a hybrid cultivar of 34%, increasing sugars and proteins in bulbs. The yield gain was not related to nutrient uptake stimuli.

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4. CAPÍTULO II - CHANGES IN THE BIOCHEMICAL ATTRIBUTES AND STORAGE OF ORGANIC ONION UNDER THE EFFECT OF APPLICATION OF MICROALGAE AND HUMIC ACID

ABSTRACT

Biostimulants are considered a sustainable technology that can be applied in the production of fresh vegetables. Microalgae arise as a new biostimulant source, as they are able to improve plant growth. However, the use of humic acid in promoting growth and improvement in the post-harvest conservation of vegetables is known; nevertheless, the possible synergy between microalgae and humic acid is still unexplored. Therefore, the influence of soaking seedlings of two onion cultivars in a solution containing the microalga *Scenedesmus subspicatus* and humic acid on the storage and biochemical characteristics of the bulbs was studied. As results, an increase occurred in fresh and dry weight in hybrid onion bulbs with positive effects on yield, reduction of in the loss of the bulb weight in open-pollinated cultivars and increased in the content of sugars, amino acids, and proteins of onions grown in an organic system.

Keywords: *Allium cepa* L.; *Scenedeusmus subspicatus*, humic substances, carbohydrates, organic farming.

1. INTRODUCTION

Onion (*Allium cepa* L.) cropping has cosmopolitan characteristics besides its notable importance worldwide. In Brazil, onion occupies the third place in economic importance among vegetables (YURI et al. 2018) with a total production of 1.6 million tons of bulbs, with an average yield of 31 tons per hectare (IBGE, 2017). Regarding food production in the organic system, especially vegetables, Brazil ranks 5th in the world ranking (MADAIL et al. 2011). Furthermore, currently, about 13% of farmers who adopt the organic production system grow onion (BRASIL, 2017).

The onion production chain has some particularities regarding postharvest participation. The incidence of bulb disease, sprouting and weight loss can economically affect marketing (CHOPE et al. 2006), also affecting farmers' income mainly due to bulb weight loss factor (RESENDE et al. 2016).

The use of more sustainable techniques in agricultural production, such as the application of products called biostimulants (for example, seaweed extracts, humic substances, amino acids), arouse increasing interest, as they interfere positively in the development of plants (DU JARDIN, 2015). Humic acid is known as a natural source of biostimulant for plants, showing potential in the metabolism of carbon and nitrogen, with positive responses on plant growth and development (CANELLAS et al. 2015).

According to Silva et al., (2011), the most reported effect of the application of humic acid on plants is the promotion of root growth. In addition to rooting, the application of humic acid to garlic plants increased productivity and reduced weight losses in storage (ABDEL-RAZZAK & EL-SHARKAWY, 2013).

Recently, the use of microalgae in the cultivation of vegetables has shown promising effects on productivity gains (MÓGOR et al. 2018b). The use of the microalga *Acutodesmus dimorphus* in tomato and *Scenedesmus* sp. in Petunia showed a capability in promoting plant growth (GARCIA-GONZALEZ & SOMERFIELD, 2016; PLAZA et al. 2018). In addition, Mógor et al. (2018a) identified an increase in the concentrations of soluble sugars, free amino acids and soluble proteins as a result of leaf suspension applications of the microalga *Arthrospira platensis* in beet.

Studies on reduction in storage losses in vegetables were carried out using biostimulants based on marine macroalgae (KHAN et al. 2018), amino acids (D'ANGELO et al. 2017) and humic substances (SHEHATA et al. 2017). However, works related to the use of microalgae and their influence on the storage of vegetables are scarce, and the potential synergistic action of microalgae and humic substances is still unexplored.

Hence, the objective of this work was to identify the effect of immersion of onion seedlings at the time of planting in solutions containing biomass of *Scenedesmus subspicatus* microalgae associated with humic acid on growth, storage and biochemical changes in onion bulbs of two cultivars produced in the organic system.

2. MATERIAL AND METHODS

2.1 Field experiment

Biomass of the microalgae *Scenedesmus subspicatus* (Sc) Chodat (synonym: *Desmodesmus subspicatus*) was obtained using the strain provided by the "Elizabeth Aidar" Microalgae Collection owned by Fluminense Federal University, Niteroi, Rio de Janeiro - Brazil. The autotrophic axenic cultivation was carried out in a semi-continuous cultivation system in a photobioreactor using WC culture (GUILLARD & LORENZEN, 1972) medium maintained at constant temperature (20-22ºC) and light (5500 lux), at the Crop Sciences and Plant Protection Department of the Federal University of Paraná, Paraná State, Brazil. After 25-day cultivation, the biomass was separated from

the culture medium by centrifugation, attaining 0.95 $g.L^{-1}$ DW and then it was lyophilized, thus being ready to use.

The commercial formulation of humic acid (HA) used in the experiment contained 33% of organic carbon obtained by extraction, grinding, precipitation and filtration of the mineral Leonardite (Powhumus® Humintech GmbH – Germany).

The experiment was conducted on the Organic Vegetables Production Research Area, where an organic system was implemented 13 years ago at the Federal University of Paraná, under the geographical coordinates 25° 25 S and 49° 06′W at an altitude of 920 m. According to Köppen classification, the climate in the area was temperate type Cfb. Chemical analysis of the 0–20 cm layer of soil in the field indicated the average values: pH 6.30 (H_2O), 33.30 $g.dm^{-3}$ organic matter; 133.10 $mg.dm^{-3}$ P; 1.44 $cmolc.dm^{-3}$ K; 9.30 $cmolc.dm^{-3}$ Ca; 4.30 $cmolc.dm^{-3}$ Mg; 0 cmolc.dm. Al; 3.7 cmolc.dm. Al+H; 18.34 $cmolc.dm^{-3}$ CEC and 80% base saturation. Seven days prior to transplanting, the soil was prepared with the incorporation of 8 $t.\ ha^{-1}$ organic compost with the following average values: C = 30.3 $g\ kg^{-1}$; N = 30.3 $g\ kg^{-1}$; P = 8.5 $g\ kg^{-1}$; K = 6.6 $g\ kg^{-1}$; Ca = 8.1 $g\ kg^{-1}$; Mg = 4.1 $g\ kg^{-1}$. The soil fertilization was done according to the Brazilian regulation for organic agriculture.

In May 2017, ‘BR-29’ (open-pollinated cultivar – ‘B’) and ‘Perfecta F1’ (hybrid – ‘P’) (Topseed®) onions, both commonly used by farmers in Southern Brazil were sowed in a nursery type seedbed under polyethylene tunnel. At 45 days after sowing (DAS), the seedlings displaying five leaves, average pseudostem diameter of 3.08 mm (‘B’) and 4.32 mm (‘P’) and properly developed roots were collected and used for field experiment. The onion seedlings were transplanted on beds with a dimension of 1.20 x 24 m, with the spacing of 30 cm between rows and 10 cm between plants, distributed in 4 planting rows, equivalent to a plant population around 230000 per hectare.

Treatments consisted in soaking roots of onion seedling for one minute in three differeent solutions: distiled water (control), 0.3 $g.L^{-1}$ HA + 0.3 $g.L^{-1}$ Sc (3SH) and 0.6 gL^{-1} HA + 0.6 gL^{-1} Sc (6SH).

At 150 days after transplanting, it was observed that about 80% of the plants had their aerial part lying close to the ground (lodging), allowing the harvesting. Nine commercial bulbs (35 to 70 mm) were stored in plastic boxes and then in a room with an average temperature of 23.5°C and relative humidity of 74% ± 5.

The fresh (FW) and dry (DW) weight of the bulbs, the ratio between fresh weight and dry weight and weight loss (WL) (Equation 1) of the bulbs of both cultivars were evaluate (every fortnight up to 60 days of storage).

$$WL(\%) = \frac{(W_i - W_f)}{W_i} \cdot 100 \quad (1)$$

Where:

W_i= initial weight; and

W_f= final weight.

2.2 Biochemical analyses

At the end of the storage period, the concentration of total sugars, reducing sugars, total free amino acids and soluble proteins were determined. For the quantification of total sugars, 1 ml of homogenized sample was collected and then added with 1 ml of HCl for acid hydrolysis in a water bath (100°C) for 10 minutes. Then, 1 ml of NaOH solution was added into it and allowed to rest for 5 minutes. After this procedure, it was added to the 3,5-Dinitrosalicylic acid (DNS) solution in the sample of total sugars and reducing sugar and reading was performed in a spectrophotometer. The standard curve for reducing and total sugars was made with 1 mg/mL (5.5 mM) glucose with ranging from 50 to 800 µg / mL (MALDONADE et al. 2013).

Total free amino acids were extracted according to Winters et al. (2002), and the colorimetric reaction was performed according to Magné and Larher (1992) using glutamine for the standard curve. Therefore, in a test tube containing the sample and distilled water, they were conditioned in the water bath 100 °C for 15 minutes, which allowed sample cooling and decanting for 20 minutes in an ice bath. After that, the samples were collected and centrifuged at 3000 rpm for 10 minutes. One milliter of the sample, 0.5 ml of citrate buffer, 1 ml of ninhydrin were added into the colorimetric reaction and shaken for 2 seconds. The water bath was also used for 15 minutes at 100 ° C, and then it was allowed to cool by adding 60° alcohol and spectrophotometer readings were carried out.

Soluble proteins were determined by adopting the methodology described by Bradford (1976) using BSA for the construction of the standard curve. For extraction of the proteins in the sample (0.5 ml), 1.5 ml of phosphate buffer was added as described by Du et al. (2010). Then, the sample was centrifuged at 10,000 rpm for 15 minutes. After that, 70 µl of the supernatant plus 2 ml of the Bradford reagent was then added and left for rest for 15 minutes, so, the sample could have been read in a spectrophotometer.

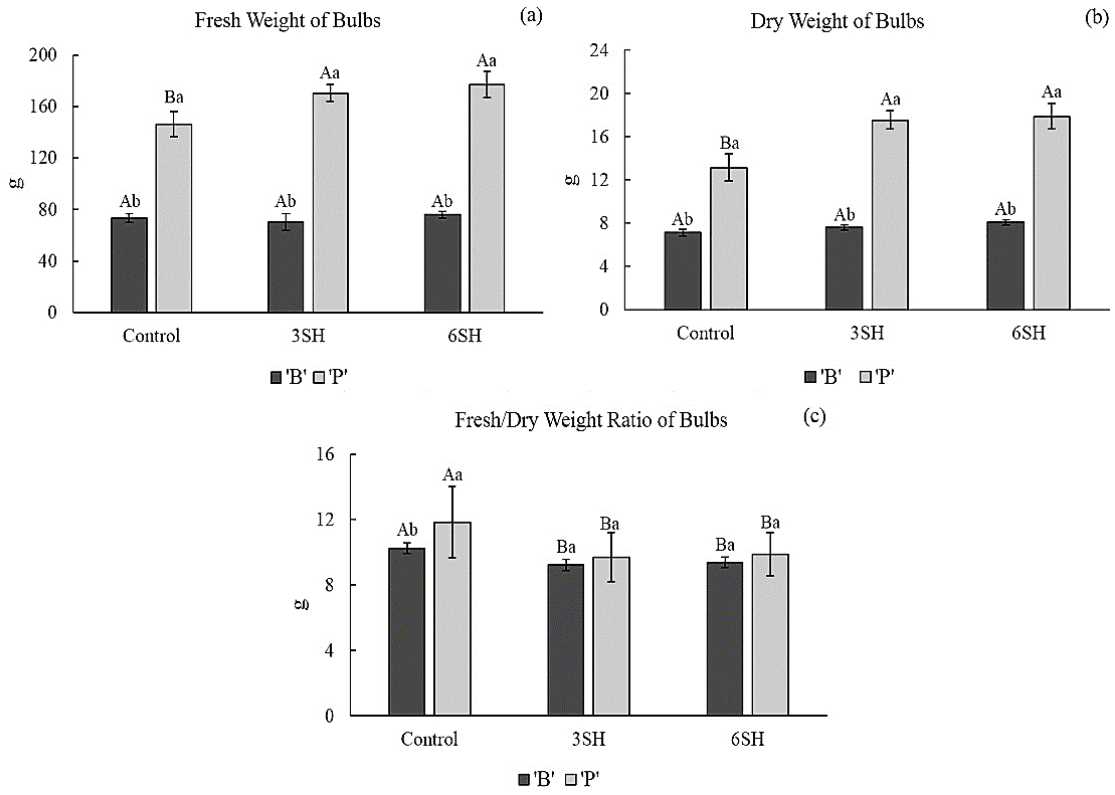
2.3 Statistical analysis

The experiment was conducted in a completely randomized design in a factorial scheme, with three factors. Factor A was the effect of the responses of the cultivars, factor B was the effect of treatments (control, 3SH, and 6SH) and factor C was the time in days, with four replicates for the weight loss variable (%). For the biometric variables (fresh weight, dry weight and ratio) and biochemical variables (sugars, amino acids, and proteins), the factorial scheme was adopted with two factors, where factor A was the effect of the responses of the cultivars and factor B was the effect of the treatments (control, 3SH and 6SH) with four replicates. The data were submitted to the Bartllet test to verify the homogeneity of the variances. Then, the ANOVA was performed using the Scott-Knott mean test at 5% probability.

3. RESULTS

Data on the fresh and dry weight of the bulbs (Fig. 1ab) showed a factor interaction between the cultivars and the treatments via immersion. The Perfecta F1 (‘P’) bulbs showed higher fresh and dry weight when compared to BR-29 (‘B’) bulbs. By comparing the treatments, the concentrations of 3SH and 6SH promoted a higher fresh and dry weight of ‘P’ bulbs than control, which was not observed in ‘B’ (Fig. 1ab). For the variable of the fresh and dry weight ratio (Fig. 1c), a factorial interaction occurred between cultivars and treatments. The highest values found in the fresh and dry weight ratio were found in ‘P’ bulbs in the control treatment followed by ‘B’ also in the control treatment. The application of SH (Humic acid plus *Scenedesmus subspicatus*) provided lower values of fresh and dry weight ratio when compared to control treatments, being statistically the same among cultivars.

FIGURE - 1 EFFECT OF SOAKING ROOTS IN SH (HUMIC ACID PLUS *Scenedesmus subspicatus*) TREATMENT ON FRESH (A), AND DRY WEIGHT (B) AND FRESH/DRY WEIGHT RATIO OF BULBS (C) OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND BARS THE STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. LOWERCASE LETTERS = CULTIVARS. UPPER CASE LETTERS = TREATMENTS



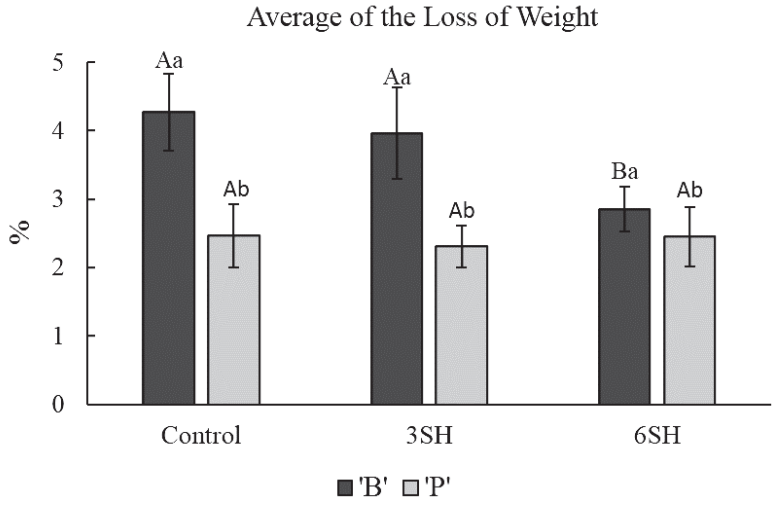
A factorial interaction was observed between cultivars, the interval between evaluations (15 days) and treatments on the loss of the weight (%) (Table 1). When the loss of weight over time was compared, it was found that the greatest losses in ‘B’ occurred at 15 and 60 DOS (Days of storage) in the control and 3SH treatments. The 6SH treatment, at 15 days, presented greater weight loss when compared to the other fortnights, however, at 30, 45 and 60 DOS, the losses were statistically the same. In ‘P’, the largest weight losses occurred in the first 15 DOS, regardless of the treatments, where the weight of the bulbs remained without change up to 60 DOS (Table 2).

TABLE 1 - CHANGES IN LOSS OF WEIGHT (%) OF BULBS OF TWO CULTIVARS TREATED WITH SH (HUMIC ACID PLUS *Scenedesmus subspicatus*) THROUGH SOAKING CONDUCTED UNDER ORGANIC PRODUCTION SYSTEM. VALUES REPRESENT THE MEANS FOLLOWED BY THE STANDARD DEVIATION. DIFFERENT LETTERS DENOTE SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. LOWERCASE LETTERS = COLUMNS. UPPER CASE LETTERS = LINES

		Days				
		15	30	45	60	∑ (%)
'B'	Control	5.41 ± 0.82 Aa	3.92 ± 0.57 Ba	2.15 ± 0.19 Ca	5.59 ± 0.67 Aa	17.09
	3SH	5.45 ± 0.47 Aa	3.02 ± 0.68 Bb	2.27 ± 0.68 Ca	5.09 ± 0.83 Aa	15.85
	6SH	5.27 ± 0.79 Aa	1.99 ± 0.30 Bc	1.68 ± 0.31 Ba	2.46 ± 0.25 Bb	11.41
'P'	Control	4.73 ± 0.62 Ab	1.92 ± 0.54 Bc	1.34 ± 0.17 Ba	1.85 ± 0.49 Bc	9.85
	3SH	4.26 ± 0.51 Ab	1.63 ± 0.10 Bc	1.85 ± 0.20 Ba	1.46 ± 0.42 Bc	9.22
	6SH	4.66 ± 0.76 Ab	1.87 ± 0.31 Bc	1.78 ± 0.29 Ba	1.48 ± 0.25 Bc	9.80

The averages of loss of the weight of bulbs are shown in Figure 2, indicating an interaction between cultivars and treatments. Among the cultivars, the ‘P’ bulbs had a lower weight loss when compared to ‘B’ bulbs. By comparing the treatments, no effect was observed in ‘P’; however, in ‘B’, there was a significant difference, in which 6SH provided a reduction in the loss of the weight by 33% when compared to the control.

FIGURE 2 - EFFECT OF SH (HUMIC ACID PLUS *Scenedesmus subspicatus*) TREATMENT UTILIZING SOAKING ON THE AVERAGE OF THE LOSS OF WEIGHT (%) OF BULBS OF TWO ONION CULTIVARS CONDUCTED IN AN ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND BARS ARE THE STANDARD DEVIATIONS. DIFFERENT LETTERS DENOTE SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. LOWERCASE LETTERS = CULTIVARS. UPPER CASE LETTERS = TREATMENTS



Biochemical changes at the end of the storage period were found when comparing cultivars and treatments (Table 2). The ‘B’ bulbs showed a higher total sugar concentration in the mean (59.6%) when compared to ‘P’. The SH treatments provided a significant increase in total sugars in both cultivars, with a gain of 16.2% for ‘B’ bulbs and 11.2% for ‘P’ bulbs. A higher concentration of reducing sugar was found in ‘P’ bulbs (93%) when compared to ‘B’ bulbs. On the other hand, ‘B’ plants that

received SH treatment at both concentrations accumulated 33% more reducing sugar when compared to the control. As for the 'P' bulbs, the 6SH treatment raised the sugar content by approximately 20.7% when compared to the other treatments.

Total free amino acids in the bulbs showed an interaction between cultivars and treatments (Table 2). Higher concentrations of total free amino acids were quantified in 'B' when compared to 'P'. The application utilizing 6SH soaking significantly increased the total free amino acids in 'B' (14%). No significant difference between treatments was found in 'P'.

The levels of soluble proteins in bulbs also showed significant differences between treatments and cultivars (Table 2). The 'B" bulbs accumulated 42% more protein than 'P'. The application of 3SH provided a greater accumulation of soluble protein (83%) in 'B' when compared to the control. On the other hand, 'P', when submitted to 6SH treatment, presented 30% more soluble proteins when compared to the control bulbs.

TABLE 2 - CHANGES IN TOTAL SUGARS (A), REDUCING SUGAR (B), TOTAL FREE AMINO ACIDS (C) AND SOLUBLE PROTEINS (D) OF BULBS OF TWO ONION CULTIVARS TREATED WITH SH (HUMIC ACID PLUS *Scenedesmus subspicatus*) UTILIZING SOAKING CONDUCTED IN AN ORGANIC SYSTEM. VALUES REPRESENT THE MEANS FOLLOWED BY THE STANDARD DEVIATION. DIFFERENT LETTERS DENOTE SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. LOWERCASE LETTERS = COLUMNS. UPPER CASE LETTERS = LINES

(a) Total sugars (mg.g^{-1})			
	Control	3 SH	6 SH
B	203.20 ± 12.50 Ba	260.90 ± 12.46 Aa	250.23 ± 13.08 Aa
P	131.99 ± 8.91 Bb	158.94 ± 4.45 Ab	166.87 ± 9.16 Ab
(b) Reducing sugar (mg.g^{-1})			
	Control	3 SH	6 SH
B	208.8 ± 21.50 Bb	289.7 ± 19.46 Ab	266.3 ± 15.40 Ab
P	418.13 ± 14.98 Ca	489.47 ± 4.81 Ba	541.8 ± 36.53 Aa
(c) Total free amino acids (mg.g^{-1})			
	Control	3 SH	6 SH
B	121.81 ± 14.18 Ba	114.01 ± 11.68 Ba	141.43 ± 10.61 Aa
P	42.33 ± 1.29 Ab	50.68 ± 4.27 Ab	46.01 ± 3.33 Ab
(d) Soluble protein (mg.g^{-1})			
	Control	3 SH	6 SH
B	76.01 ± 8.63 Ba	139.16 ± 15.80 Aa	85.67 ± 6.22 Ba
P	62.84 ± 7.91 Ba	67.01 ± 4.75 Bb	81.39 ± 10.55 Aa

4. DISCUSSION

Advances in the study on the improvement of new onion cultivars, especially hybrid materials, have demonstrated greater production capacity, homogeneity of production in the field and better conservation in storage, with higher acceptance of the consumer market due to the production of bulbs of larger size (YURI et al. 2018). Thus, bulbs with an average weight of 150 g show greater commercial preference and direct monetary gains to producers (KURTZ et al. 2018).

The gains in fresh weight and dry weight (Table 1a, b) of the hybrid cultivar bulbs when treated with SH at both concentrations may be partly explained by the plant growth promoting effect of humic acid. The highest expression of the H+ATPase enzyme is stimulated by the presence of humic acid in vegetables, which promotes plant development, an effect similar to that of auxin phytohormone (JINDO et al. 2012). The ability to promote plant growth was also verified when microalgae were applied in plants, and this effect is attributed by the presence of biomolecules such as polyamines and cytokinins (MÓGOR et al. 2018b; STIRK, 2013).

The ratio between fresh and dry weight of the bulbs (Table 1c) indicates that lower water contents in the bulbs are directly linked to the lower values of the ratio, thus, bulbs treated with SH in both cultivars show lower water content when compared to the control, indicating greater accumulation of photoassimilates in the bulbs. The reduction in water content in the bulbs can be explained by the promotion in cell division caused by humic acid and microalgae, which provides an increase in biomass in the most important drains (BETTONI et al. 2016; PLAZA et al. 2018).

Kahsay et al. (2013) indicate that lower losses of weight in bulbs are associated with a more significant accumulation of dry weight and sugars. This result is similar to that found in this study, where bulbs of the hybrid cultivar (‘P’) display lower loss of weight when compared to ‘B’ over the 60 days of evaluation (Table 2). So, this effect may be related to a greater accumulation of dry weight in P, indicated by the relation between fresh/dry weight in this cultivar (Fig 1c) (Fig. 2).

Shehata et al. (2017), observed a positive effect of the action of humic acid and macroalgae extract on the production and reduction of post-harvest losses in onion. According to the authors, the greater accumulation of dry weight associated with the treatments caused losses in the weight of the bulbs with lower intensity when compared to the control. Among the cultivars, this effect was observed, where ‘P’ bulbs

had a greater dry weight (Table 1b) than the ‘B’ bulbs, which resulted in lower weight losses.

However, the dry weight in ‘B’ was the same among treatments (Fig. 1b), but the lower weight loss was observed in plants treated with 6SH (Table 1) when compared to the other treatments. When the ratio between fresh and dry weight (Fig 1c) was analyzed, SH-treated plants showed a greater accumulation of photoassimilates, which may have caused less weight loss. Nevertheless, even if plants treated with 3SH and 6SH had accumulated more biomass (Fig. 1c), only 6SH treatment provided less weight loss.

Zang et al. (2016), show that in the dry matter fraction of onion bulbs, approximately 80% of it is composed of non-structural carbohydrates, which are reducing sugars, glucose, and fructose, as well as non-reducing sugar, sucrose, and fruits- oligosaccharides. According to Kahsay et al. (2013), smaller losses in onion bulb weight during the storage period is related to higher carbohydrate concentrations, a result similar to that found in this study, where ‘P’ bulbs that showed higher sugar concentration lost less weight than ‘B’ bulbs. It was verified that the ºBrix values of onion bulbs between treatments were statistically equal ($p > 0.05$). However, bulbs from cultivar 'P' showed higher ºBrix values (44%) when compared to 'B' bulbs. The average ºBrix values for “B’ and 'P' respectively are: control = 5.17 and 8.0; 3SH = 6.25 and 8.05; 6SH = 5.57 and 8.45.

Moreover, studies with humic acid application in onion plants show an increase in the concentration of reducing sugars and starch in the bulbs (BETTONI et al. 2016), as well as the microalgae *Nannochloropsis* sp. which once applied in tomato plants also increased carbohydrates (COPPENS et al. 2016). These results corroborate the effect of the 6SH treatment applied in ‘B’, with the association of microalga and humic acid promoting a greater accumulation of reducing sugars (Table 2), resulting in a decrease in the loss of weight (Table 1).

Few studies discuss the influence of amino acid content in bulbs and their relationship to storage conservation (SHARMA et al. 2015). In this study, the ‘B’ bulbs that had the highest concentration of total free amino acids lost more weight when compared to ‘P’ bulbs (Table 2).Immersion with SH showed the potential for promoting the increase in soluble proteins in the onion bulbs (Table 2); however, without any relation to the weight loss of the bulbs over storage.

Further investigations may deepen the study of the effects of SH signaling on plants on the accumulation of sugars in the drains and the impacts on the reduction in loss of weight in onion bulbs.

5. CONCLUSION

The treatment of onion seedlings with humic acid combined with *Scenedesmus subspicatus* microalgae promoted a greater accumulation of photoassimilates in the bulbs of two onion cultivars with a reduction in the loss of the weight over 60 days of storage of the open-pollinating cultivar. The treatments provided in both cultivars an increase in sugars and total proteins in the bulbs, and an increment in amino acids in the open pollinated cultivar.

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5. CAPÍTULO III – ORGANIC ONION GROWTH, YIELD AND STORAGE IMPROVED BY FOLIAR SPRAYS OF MICROALGAE AND FULVIC ACID AS A NATURAL BIOSTIMULANT

ABSTRACT

The use of microalgae as natural biostimulants in horticulture has recently been reported, while the use of humic substances is widespread. However, the combined use of microalgae with humic substances applied to plant leaves is still unexploited. Thus, the objective of this work was to combine fulvic acid (FA) with the *Scenedesmus subspicatus* microalgae biomass (SC) as a natural biostimulant applied via leaf in two onion cultivars in organic system. Four experiments were conducted: i) bioassays to verify the bioactive effect of FA, SC and combinations using the *Vigna radiata* model plant; ii) greenhouse pot experiment with foliar applications of FA, SC and combination in two onion cultivars; iii) field experiment in organic system with foliar applications of FA, SC and combination in two onion cultivars; and iv) onion bulb storage experiment. The bioactive effect of SC, FA and their combinations was identified by promoting changes in root growth of *V. radiata*. In pots, treatments containing FA, SC and combination promoted increase in fresh and dry leaf mass. The foliar application of FA, SC and combination promoted an increase in field bulb productivity, reduced mass loss in stored bulbs and increased carbohydrate, amino acid and protein contents in onion bulbs.

Keywords: *Allium cepa* L.; Biostimulants; Humic substances; organic farming; *Scenedesmus subspicatus*.

1. INTRODUCTION

The production of foods free of synthetic substances has been valued by consumers, causing farmers to migrate to alternative production systems such as organic farming (MADAIL et al. 2011). As a result, the development of sustainable technologies, suitable for the organic system and providing productivity and storage gains has become strategic.

Biostimulants are a sustainable technology, composed of natural substances or microorganisms that provide plants with gains in productivity (DU JARDIN, 2015). Kelp extract and humic substances (HS) are reported as biostimulant sources, and recently, the use of microalgae in horticulture showed effect on promoting plant growth as well as increasing productivity (MÓGOR et al. 2018b).

Estimates indicate the existence of 800,000 microalgae species, and only about 6.25% have been described (SUGANYA et al. 2016). Recent studies approach the potential of using microalgae, as these unicellular organisms present compounds of

interest to the biofuel, pharmaceutical, animal and human nutrition industries (ISHAQ et al. 2016). In addition, phytohormones such as auxins and cytokines were found in microalgae *Arthrospira platensis* and *Scenedesmus almeriens*. Such hormones when applied to plants provided significant increase in root growth (PLAZA et al. 2018).

Studies using *Arthrospira platensis* in lettuce (MÓGOR et al. 2018a), *Acutodesmus dimorphus* in tomato plants (GARCIA-GONZALEZ, SOMMERFELD 2016), *Scenedesmus subspicatus* in onion (GEMIN et al. 2019) and in beet (BARONE et al 2017) identified the growth promoting effect of these microalgae.

In addition to microalgae, the most studied fractions of HS, humic and fulvic acids also have an effect on promoting plant growth and development (CANELLAS et al. 2015). When studying the foliar application of HS in onion cultivation, Bettoni et al. (2017) observed the increment in total solids concentration, as well as a gain of fresh and dry mass in bulbs. This effect on plant growth and development can be partly explained by the positive effect of humic substances on plant photosynthetic metabolism and by the stimulation of the enzyme H+ATPase, an effect similar to phytohormones auxin (CANELLAS; OLIVARES, 2014).

The use of humic acid and microalgae applied via leaves results in the promotion of plant growth with productivity gains, greater accumulation of sugars in plants and reduction in mass loss over storage (BETTONI et al. 2016, MÓGOR et al. 2018a; SHEHATA et al 2017). The combination of humic acid and microalgae was recently reported in a study of onion seedlings immersion with significant yield gains in the organic system (GEMIN et al. 2019). However, the foliar use of the combination of fulvic acid and microalgae is still unexplored.

Therefore, the objective of this work was to evaluate the effect of foliar applications of fulvic acid and *Scenedesmus subspicatus* microalgae biomass, alone or in combination through the implementation of four experiments: (i) bioassay using *Vigna radiata* L. model plant to identify bioactivity of microalgae biomass (SC), fulvic acid (FA) and their combinations (FS) in promoting root growth; (ii) initial growth of onion plants grown in pots in a protected environment submitted to foliar applications of FA, SC and FS; (iii) field experiment in organic system evaluating the effect of FA, SC and FS foliar applications on yield and biochemical alterations in bulbs of two cultivars; and (iv) influence of foliar application of FA, SC and FS on onion plants on mass loss and biochemical alterations of bulbs stored for 60 days.

2. MATERIAL AND METHODS

The fulvic acid (Fulvital® wsp80) Humin tech (GmbH - Germany) used in the experiment is a dry powder extracted from the mineral Leonardite with high water solubility and suitable for use in organic agriculture (EU Reg.EC 834/2007 and 889 / 2008).

The microalgae *Scenedesmus subspicatus* Chodat (synonym: *Desmodesmus subspicatus*) biomass was obtained in an autotrophic axenic cultivation performed in semi-continuous photobioreactor, using WC culture medium maintained at constant temperature (20–22°C) and light (5500 lx), at the Crop Sciences and Plant Protection Department of the Federal University of Paraná. After 25-day cultivation period, the biomass was separated from the culture medium through centrifugation, attaining 0.95 g L^{-1} DW and then it was lyophilized. The strain was provided by the "Elizabeth Aidar" Microalgae Collection from the Fluminense Federal University, Niteroi, Rio de Janeiro - Brazil.

2.1 Bioassays

Bioassays were conducted with *Vigna radiata* L. using growth chambers with controlled temperature and light (22ºC; photon flux intensity: 0.52–0.56 mmol/m^2 /sec) for 15 days (MÓGOR et al,. 2017). The objective was to evaluate the possible bioactivity of SC (first step) and FA and of combinations of SC (second step) in root growth.

The treatments used to test the microalga SC lyophilized biomass were (first step) the following: control with distilled water, 0.25, 0.50, 0.75 and 1.00 g.L^{-1} SC. The treatments using FA and combinations with SC were (second step) the following: distilled water control, 0.05 gL^{-1} FA; 0.10 gL^{-1} FA; 0.15 gL^{-1} FA; 0.2 gL^{-1} FA; 0.05 gL^{-1} FA + 0.5 gL^{-1} SC; 0.10 gL^{-1} FA + 0.5 gL^{-1} SC; 0.15 gL^{-1} FA + 0.5 gL^{-1} SC and 0.20 gL^{-1} FA + 0.5 gL^{-1} SC.

Average root length (sum of the length of all roots) and root average volume were determined using the Winrhizo Pro® software (Regent Instr. ® Canada) coupled with an Epson® double lens scanner (V700 PHOTO model).

A completely randomized design was used in the first step; five treatments were used and in the second, nine treatments, both with 4 repetitions containing 8 plants per repetition. Data were submitted to test of Bartlett and ANOVA tests. When

significant, the mean test adopted was Scott-Knott ($p < 0.01$) using the Assistat 7.7 beta statistical software.

2.2 Pot experiment

Based on the results obtained in the bioassay, concentrations of FA, SC and combination (FS) were defined to conduct the experiment in a greenhouse. Direct sowing was carried out in plastic pots (3 L) of two onion cultivars, one open pollinated ('B' - cv. BR-29) and the other hybrid ('P' - cv. Perfecta F1) both from Topseed ®. The pots were filled with soil suitable for onion cultivation, with: 5.84 pH (H_2O); 26.31 $g.dm^{-3}$ organic matter; 49 $mg.dm^{-3}$ P; 1.32 $cmolc.dm^{-3}$ K; 5.28 $cmolc.dm^{-3}$ Ca; 3.05 $cmolc.dm^{-3}$ Mg; 0 $cmolc.dm^{-3}$ Al; 2.93 $cmolc.dm^{-3}$ Al+H; 12.58 $cmolc.dm^{-3}$ CEC and 76.7% of base saturation.

The treatments consisted of solutions containing the control with distilled water, 0.2 $g.L^{-1}$ FA, 0.5 $g.L^{-1}$ SC and combination with 0.2 $g.L^{-1}$ FA + 0.5 $g.L^{-1}$ SC (FS), applied weekly totaling 6 applications. A Kawashima electric sprayer (model Kws Pem-P20) was used, maintaining constant pressure (2 bar) in the applications, resulting in a spray volume of 60 mL per repetition.

To identify the effect of the applications on the initial growth, the aerial part of the onion plants was collected after 60 days of sowing (DAS). Data of fresh mass, dry mass and fresh/dry mass ratio of the aerial part of both cultivars were obtained using a two-digit digital scale after the dots.

The experimental design was completely randomized in a factorial scheme (n = 4) (factor A was the cultivars; factor B the treatments), each repetition consisting of two pots containing three plants per pot. The average data per plant were submitted to Bartlett and ANOVA tests. The mean test adopted when significant was Scott-Knott ($p < 0.05$) using the Assistat 7.7 beta statistical software

2.3 Field experiment

The experiment was carried out in the organic garden area of the Federal University of Paraná (UFPR), where the organic production system has been adopted for over a decade. The climate of the region according to the Köppen classification is Cfb. The chemical composition of the soil shows the following average values: 6.30 pH (H_2O), 33.30 $g.dm^{-3}$ organic matter; 133.10 $mg.dm^{-3}$ P; 1.44 $cmolc.dm^{-3}$ K; 9.30

cmolc.dm^{-3} Ca, 4.30 cmolc.dm^{-3} Mg; 0 cmolc.dm^{-3} Al; 3.7 cmolc.dm^{-3} Al+H; 18.34 cmolc.dm^{-3} CEC and 80% base saturation. One week prior to onion seedling transplantation, soil preparation was carried out with the incorporation of organic compost at a dose of 8 tons per hectare, whose chemical characteristics were the following: C = 30.3 g.kg^{-1}; N = 30.3 g.kg^{-1}; P = 8.5 g.kg^{-1}; K = 6.6 g.kg^{-1}; Ca = 8.1 g.kg^{-1} and Mg = 4.1 g.kg^{-1}.

Seedling production was carried out in beds protected by a polyethylene tunnel, where the same onion cultivars (‘B’ and ‘P’) of the pot experiment were sown. After 45 sowing (DAS) the seedlings were ready for transplanting, with an average of 5 leaves, with average pseudostem diameter of 10.94mm and 13.62mm for ‘B’ and ‘P’ respectively. The onion seedlings were transplanted into beds with a size of 1.20 x 48 m, with a spacing of 30 cm between rows and 10 cm between plants, distributed in 4 planting rows, equivalent to a plant population of 230,000 per hectare

The treatments were the same as those used in the pot experiment. Applications began simultaneously with the beginning of bulb development (90 DAT). Seven foliar applications were performed at weekly intervals, ending at 140 DAT. An electric sprayer (Kawashima – model Kws Pem-P20) with a constant pressure of 3 bar was used, and a spray volume equivalent to 600 L.ha^{-1} per application was used for each treatment.

2.3.1 Biometric and yield analysis

At 150 days after transplanting, about 80% of the plants showed collapse of the pseudostem, thus indicating the proper harvest time. Eight onion plants were collected from each repetition, separating the leaves from the bulbs. Following this, the fresh mass was measured using a digital scale. For the dry mass, the bulbs were cut into 8 parts, placed in paper bags and placed in an oven at 60 °C with forced ventilation until they reached constant weight. Then, the bulb dry mass was measured, allowing the calculation of the fresh and dry mass ratio. Yield data were calculated according to the average fresh bulb mass per treatment extrapolated per hectare for a population of 230,000 plants.

Bulb calibers were classified according to the Brazilian commercial classification (MAPA - Ministry of Agriculture, Livestock and Supply), which classifies the bulbs into the following types: type 1 (<35mm), type 2 (35 to 50 mm), type 3 (50 to 70 mm), type 4 (70 to 90 mm) and type 5 (> 90mm).

2.3.2 Storage of bulbs for 60 days

To verify the mass loss of the bulbs over storage, 8 bulbs of each repetition per treatment (control, FA, SC and FS) of each cultivar ('B' and 'P') were stored for 60 days. So, plastic boxes were used to pack the bulbs in a cool and ventilated place, with average temperature of 23.5°C and relative humidity of 74% ± 5. The mass loss was calculated using equation 1:

$$WL(\%) = \frac{(W_i - W_f)}{W_i} \cdot 100 \quad (1)$$

Where:

$\boldsymbol{W_i}$= initial weight; and

$\boldsymbol{W_f}$= final weight.

2.3.3 Biochemical analysis

For the quantification of total sugars, reducing sugar, total free amino acids and soluble proteins of the bulbs, two bulbs of each repetition per treatment of the field experiment and 2 bulbs of each repetition per treatment after 60 days of storage were selected.

For the quantification of total sugars, 1 ml of homogenized sample was collected and then added with 1 ml of HCl for acid hydrolysis in a water bath (100°C) for 10 minutes. Then, 1 ml of NaOH solution was added into it and allowed to rest for 5 minutes. After this procedure, it was added to the 3,5-Dinitrosalicylic acid (DNS) solution in the sample of total sugars and reducing sugar and reading was performed in a spectrophotometer. The standard curve for reducing and total sugars was made with 1 mg/mL (5.5 mM) glucose with ranging from 50 to 800 µg / mL (MALDONADE et al. 2013).

Total free amino acids were extracted according to Winters et al. (2002), and the colorimetric reaction was performed according to Magné and Larher (1992) using glutamine for the standard curve. Therefore, in a test tube containing the sample and distilled water, they were conditioned in the water bath 100°C for 15 minutes, which allowed sample cooling and decanting for 20 minutes in an ice bath. After that, the samples were collected and centrifuged at 3000 rpm for 10 minutes. One millimeter of the sample, 0.5 ml of citrate buffer, 1 ml of ninhydrin were added into the colorimetric

reaction and shaken for 2 seconds. The water bath was also used for 15 minutes at 100°C, and then it was allowed to cool by adding 60° alcohol and spectrophotometer readings were carried out.

Soluble proteins were determined by adopting the methodology described by Bradford (1976) using BSA for the construction of the standard curve. For extraction of the proteins in the sample (0.5 ml), 1.5 ml of phosphate buffer was added as described by Du et al. (2010). Then, the sample was centrifuged at 10,000 rpm for 15 minutes. After that, 70 µl of the supernatant plus 2 ml of the Bradford reagent was then added and left for rest for 15 minutes, so, the sample could have been read in a spectrophotometer.

2.3.4 Statistic analysis

The experimental design used for the variables fresh mass, dry mass, fresh and dry mass ratio, yield, storage and biochemical losses was a completely randomized in a factorial scheme (n = 4) (factor A the cultivars, factor B the treatments), totaling 4 repetitions per treatment. Data were submitted to Bartlett and ANOVA tests. The mean test adopted when significant was Scott-Knott ($p < 0.05$). For the bulb caliber classification variable, the Kruskall-Wallis non-parametric test ($p < 0.05$) was used, and all statistical data were processed with the aid of Assistat 7.7 beta software.

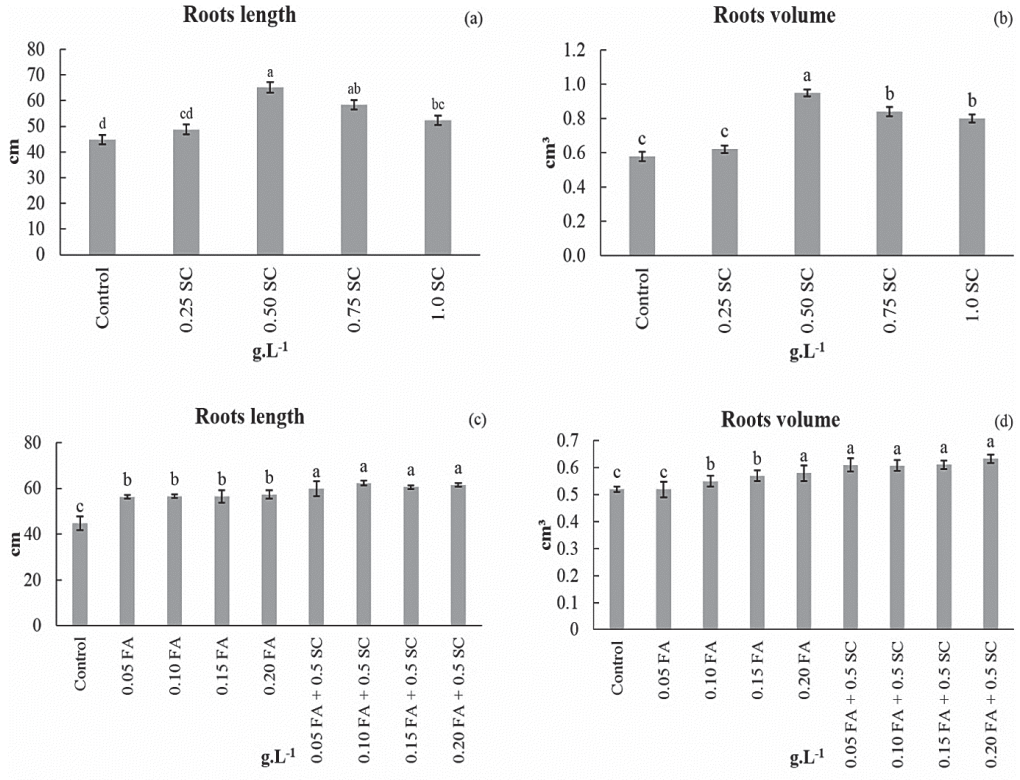
3. RESULTS

3.1 Bioassays

The results of root length and volume of *V. radiata* plants showed that the treatments containing the microalgae SC (Fig.1ab) positively influenced rooting promotion. For both variables, the concentration 0.5 $g.L^{-1}$ SC increased the average length by 45% and root volume by 66% compared to the control. Root length promotion was also observed among treatments containing FA (Fig. 1c), on average showing 28% longer roots when compared to control. However, the combination of SC (0.5 $g.L^{-1}$) with the different FA concentrations potentiated the root length by 36% when compared to the control (Fig. 1c).

For root volume (Fig. 1d), the lowest dose of FA (0.05 $g.L^{-1}$) did not differ statistically from the control. However, 0.10 and 0.15 $g.L^{-1}$ of FA showed significant gain in root volume when compared to control. Moreover, 0.2 gL^{-1} FA treatment and combinations containing concentrations of 0.05, 0.1, 0.15 and 0.20 $g.L^{-1}$ FA + 0.5 $g.L^{-1}$ SC showed better effect, increasing root volume by 16.5% on average when compared to control.

FIGURE 1 - AVERAGE VALUES OF ROOTS LENGTH AND VOLUME OF *Vigna radiata* PLANTS UNDER TREATMENTS WITH: (FIGURE 1A AND 1B) CONTROL AND MICROALGAE *Scenedesmus subspicatus* (SC) LYOPHILIZED BIOMASS AQUEOUS SOLUTIONS; (FIGURE 1C AND 1D) CONTROL, FULVIC ACID (FA) AND FULVIC ACID PLUS *Scenedesmus subspicatus* (FS) LYOPHILIZED BIOMASS AQUEOUS SOLUTIONS



3.2 Pots experiment

Among cultivars, ‘P’ plants presented on average higher fresh and dry mass when compared to ‘B’ plants. The treatments showed significant differences in fresh and dry weight. Plants receiving FA (0.2 $g.L^{-1}$), SC (0.5 $g.L^{-1}$) and FS (0.2 $g.L^{-1}$ FA + 0.5 $g.L^{-1}$ SC) in foliar application showed an increase by 19% in fresh mass and 32% in leaf dry mass when compared to the control (Table 1).

The ratio between fresh and dry weight of the leaves among the cultivars did not present significant differences. However, it was observed that the ratio value was higher in the control treatment when compared to the other treatments, but between FA, SC and FS, they showed statistically equal values.

TABLE 1 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON FRESH AND DRY WEIGHT AND FRESH/DRY WEIGHT RATIO OF AERIAL PARTS OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND ± THE STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. ANOVA: ns = NOT SIGNIFICANT; * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY. C = CULTIVARS; T = TREATMENTS AND C X T = INTERACTIONS

Fresh Weight					
	Control	FA	SC	FS	$\bar{x}$
B'	8.03 ± 0.58	9.24 ± 0.92	9.45 ± 0.68	9.86 ± 1.55	9.15 b
P'	13.72 ± 1.97	17.30 ± 2.15	15.53 ± 1.78	16.85 ± 1.15	15.85 a
$\bar{x}$	10.88 b	13.27 a	12.49 a	13.35 a	
ANOVA					
C	**				
T	**				
C x T	ns				
Dry Weight					
	Control	FA	SC	FS	$\bar{x}$
B'	0.92 ± 0.09	1.17 ± 0.12	1.21 ± 0.93	1.26 ± 0.14	1.14 b
P'	1.63 ± 0.34	2.21 ± 0.26	1.98 ± 0.20	2.29 ± 0.16	2.03 a
$\bar{x}$	1.27 b	1.69 a	1.59 a	1.78 a	
ANOVA					
C	**				
T	**				
C x T	ns				
Ratio Fresh/Dry Weight					
	Control	FA	SC	FS	$\bar{x}$
B'	8.78 ± 0.29	7.84 ± 0.36	7.8 ± 0.47	7.81 ± 0.60	8.06 a
P'	8.5 ± 0.59	7.82 ± 0.50	7.84 ± 0.19	7.33 ± 0.20	7.87 a
$\bar{x}$	8.64 a	7.83 b	7.82 b	7.57 b	
ANOVA					
C	ns				
T	**				
C x T	ns				

3.3 Field experiment

An interaction was found between cultivar factors and treatments for fresh bulb weight (Table 2). The ‘P’ bulbs presented higher fresh weight when compared to ‘B’ bulbs. In addition, ‘P’ bulbs submitted to treatments with FA, SC and FS provided, on average, an increase in fresh weight (48%) when compared to the control. In ‘B’, the treatments also positively influenced the gain of fresh weight when compared to the control, where the largest difference was observed in plants that received SC and FS via leaf (gains of 50% and 47% respectively).

Similarly, dry weight data (Table 2) also showed interaction between cultivars and treatments. ‘P’ bulbs showed higher dry weight values compared to ‘B’ bulbs. The application of FS in ‘P’ provided higher values of dry weight when compared to the other treatments, twice as much as the control. The FA and SC treatments in ‘P’ promoted gains by 45% and 85% in dry mass compared to control. For ‘B’ bulbs, the treatments SC and FS increased the dry weight of the bulbs by 33% and 36% respectively when compared to the control. The FA treatment in ‘B’ bulbs presented statistically dry mass equal to the control.

The values of fresh and dry weight ratio (Table 2) in the bulbs showed interaction between cultivars and treatments. In ‘B’, the treatments did not influence the ratio values, however, the control, FA and SC treatments had lower ratio values when compared to the same treatments used in ‘P’. For the FS treatment, in both cultivars, they presented equal ratio values. Moreover, in ‘P’ bulbs, the SC and FS treatments provided lower ratio values when compared to the control and FA treatments.

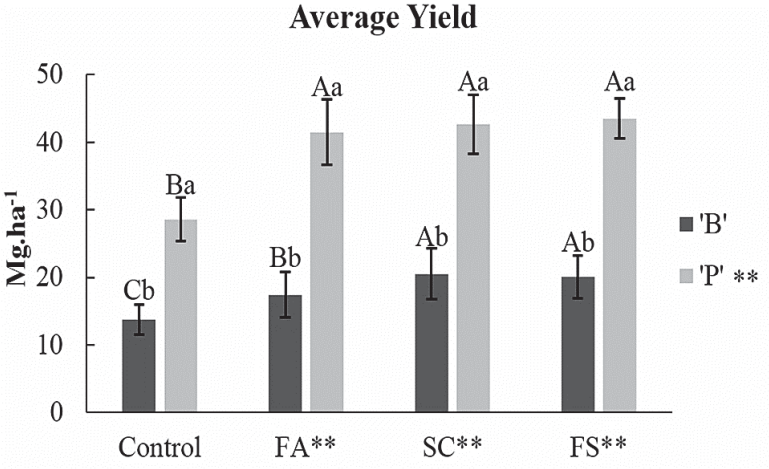
TABLE 2 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON FRESH AND DRY WEIGHT AND FRESH/DRY WEIGHT RATIO OF BULBS OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND ± STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. CAPITAL LETTERS = TREATMENTS; LOWERCASE LETTERS = CULTIVARS. ANOVA: ns = NOT SIGNIFICANT; * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY. C = CULTIVARS; T = TREATMENTS AND C X T = INTERACTIONS

Fresh Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
B'	59.92 ± 3.96 Cb	75.95 ± 2.57 Bb	89.24 ± 1.61 Ab	87.15 ± 5,17 Ab	78.07 b
P'	124.32 ± 5.13 Ba	180.29 ± 7.86 Aa	185.43 ± 6.10 Aa	189.03 ± 3.19 Aa	169.76 a
$\bar{x}$	92.12 c	128.12 b	137.34 a	138.09 a	
ANOVA					
C	**				
T	**				
C x T	**				
Dry Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
B'	6.55 ± 0.65 Bb	7.35 ±0.34 Bb	8.75 ± 0.96 Ab	8.97 ± 1.11 Ab	7.90 b
P'	8.46 ± 0.35 Da	12.34 ± 1.37 Ca	15.69 ± 1.32 Ba	17.47 ± 1.52 Aa	13.49 a
$\bar{x}$	7.51 d	9.85 c	12.22 b	13.22 a	
ANOVA					
C	**				
T	**				
C x T	**				
Ratio Fresh and Dry Weight of Bulbs					
	Control	FA	SC	FS	$\bar{x}$
B'	9.17 ± 0.30 Ab	10.34 ± 0.54 Ab	10.29 ± 1.16 Ab	9.77 ± 0.70 Aa	9.89 b
P'	14.71 ± 1.21 Aa	14.68 ±0.96 Aa	11.86 ± 0.94 Ba	10.81 ± 0.21 Ba	13.02 a
$\bar{x}$	11.94 a	12.51 a	11.08 b	10.29 b	
ANOVA					
C	**				
T	**				
C x T	**				

As observed in the results in onion bulb weight, yield data also showed significant differences between the cultivars and the treatments (Fig. 2). The cultivar ‘P’ showed on average 39 Mg/ha of bulbs, while ‘B’ presented 17 Mg/ha. Organic production of P exceeded the world average of 21 tons per hectare (FAO, 2017). The treatments influenced positively both cultivars by increasing the bulb production.

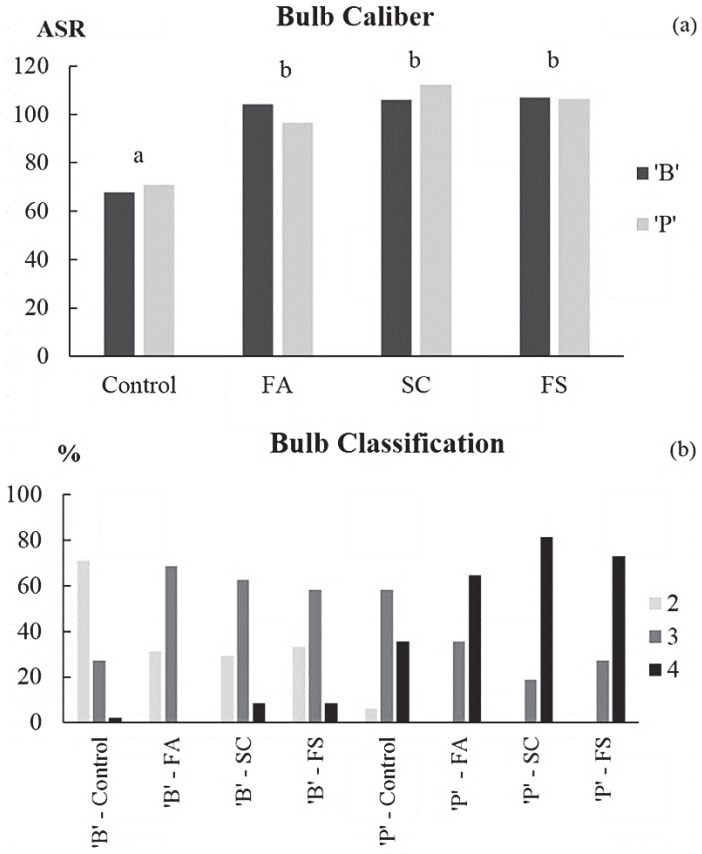
In relation to ‘P’, the foliar applications containing FA, SC and FS promoted an average yield increase (42 Mg/ha) of about 48.5% when compared to the control, which produced 28.5 Mg/ha. In ‘B’, the treatments that stood out were SC and FS, producing on average 20 Mg/ha, with an increase of 47.8% compared to the control (13.7 Mg/ha). The FA treatment increased the productivity when compared to the control in 27%; however, it was not superior to the SC and FS treatments.

FIGURE 2 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON AVERAGE YIELD OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. BARS INDICATED THE STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. CAPITAL LETTERS = TREATMENTS; LOWERCASE LETTERS = CULTIVARS. ANOVA: NS = NOT SIGNIFICANT; * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY



Regarding bulb caliber, significant differences were found between treatments (Fig 3a), where the sum of the ranks by the non-parametric test of Kruskal-Wallis indicates an increase in the number of larger caliber onion bulbs in both cultivars treated with FA, SC and FS, when compared to the control. In addition, the population of the produced bulbs fell in type 2 to 4 for both cultivars within the treatments used. The percentage of larger caliber bulbs was found in ‘P’ compared to ‘B’, which explains the superiority of ‘P’ in productivity (Fig. 3b).

FIGURE 3 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON BULB CALIBER (A) AND PERCENTAGE OF BULB PER CALIBER (B) OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. ASR = SUM OF THE RANKS OF BULBS. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE KRUSKAL-WALLIS NON-PARAMETRIC TEST BETWEEN TREATMENTS



3.4 Biochemical analysis

Interaction was found between cultivars and treatments for biochemical variables (table 3). Bulbs ‘B’ showed higher concentration of total sugars when compared to ‘P’ bulbs. Moreover, in ‘B’, the FS treatment promoted higher concentration of total sugars when compared to the other treatments, followed by SC, FA and control treatments. In ‘P’, SC application provided higher accumulation of total sugars in the bulbs, followed by FS, FA and control treatments. The largest accumulation of reducing sugar was observed in ‘P’ bulbs in comparison to ‘B’. Among the treatments, in ‘B’, the application of FS provided the largest accumulations of reducing sugar, followed by statistically equal SC and FA, where the control had the lowest concentration. In ‘P’, the application of SC and FS increased in reducing sugar accumulation in the bulbs in relation to control and FA.

Treatments did not interfere with the concentration of free total amino acids in ‘P’ bulbs. In addition, higher free total amino acid concentrations in 'B' bulbs were quantified on average. The FA, SC and FS treatments provided significant gains in free total amino acid in ‘B’ bulbs when compared to the control.

For soluble proteins, there was no significant difference between treatments in ‘B’, which in turn presented lower protein values when compared to ‘P’. In addition, the application of SC and FS raised protein concentration when compared to other treatments in ‘P’.

TABLE 3 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON TOTAL SUGARS, REDUCING SUGAR, FREE AMINO ACIDS AND SOLUBLE PROTEIN OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND ± THE STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. CAPITAL LETTERS = TREATMENTS; LOWERCASE LETTERS = CULTIVARS. ANOVA: ns = NOT SIGNIFICANT; * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY. C = CULTIVARS; T = TREATMENTS AND C X T = INTERACTIONS

Total Sugars					
	Control	FA	SC	FS	$\bar{x}$
'B'	26.09 ± 0.64 Ca	25.07 ± 0.54 Ca	29.48 ± 1.30 Ba	38.55 ± 0.98 Aa	29.80 a
'P'	13.53 ± 0.23 Db	15.63 ± 1.04 Cb	21.86 ± 0.73 Ab	19.47 ± 0.50 Bb	17.62 b
$\bar{x}$	19.81 c	20.35 c	25.67 b	29.01 a	
ANOVA					
C	**				
T	**				
C x T	**				
Reducing Sugar					
	Control	FA	SC	FS	$\bar{x}$
'B'	23.14 ± 1.13 Cb	25.47 ± 0.50 Ba	24.79 ± 0.74 Bb	31.47 ± 0.36 Ab	26.22 b
'P'	25.12 ± 0.38 Ba	26.95 ± 1.40 Ba	43.96 ± 1.06 Aa	42.97 ± 0.23 Aa	34.75 a
$\bar{x}$	24.13 d	26.21 c	34.38 b	37.22 a	
ANOVA					
C	**				
T	**				
C x T	**				
Free Amino Acids					
	Control	FA	SC	FS	$\bar{x}$
'B'	104.46 ± 3.83 Ba	173.55 ± 5.06 Aa	150.47 ± 3.01 Aa	154.04 ± 2.58 Aa	143.65 a
'P'	64.76 ± 7.70 Ab	79.71 ± 6.81 Ab	90.2 ± 8.50 Ab	94.73 ± 2.85 Ab	82.35 b
$\bar{x}$	84.61 b	126.63 a	120.33 a	124.38 a	
ANOVA					
C	**				
T	**				
C x T	*				
Soluble Protein					
	Control	FA	SC	FS	$\bar{x}$
'B'	10.14 ± 1.33 Aa	11.59 ± 0.41 Aa	10.63 ± 0.53 Aa	10.62 ± 0.41 Aa	10.74 a
'P'	9.35 ± 0.30 Ba	9.32 ± 0.33 Ba	11.63 ± 1.94 Aa	11.91 ± 0.70 Aa	10.52 a
$\bar{x}$	9.74 b	10.38 b	11.13 a	11.26 a	
ANOVA					
C	ns				
T	*				
C x T	**				

3.5 Weight loss and biochemical changes in bulb storage

Throughout the 60 days of evaluation on bulb loss, it was possible to identify a difference in weight loss by 29% more of cultivar ‘B’ compared to ‘P’ (Table 4). Treatments showed a 32% reduction in weight loss in bulbs treated with FA, 25% with SC and 30% with FS when compared to control.

In addition, at the end of the storage period, it was possible to verify biochemical changes in onion bulbs. Interaction was observed between cultivars and treatments in the variable total sugars. Bulbs of ‘B’ showed higher concentration of total sugars when compared to ‘P’ bulbs, a result similar to that found in the quantification of total sugars in bulbs collected in the field before storage. The treatments that provided the highest accumulated total sugars in ‘B’ bulbs were SC followed by the FS treatment. The FA and control treatments presented statistically equal values, being inferior to the other treatments. In ‘P’, FS treatment promoted greater accumulation of total sugars, followed by SC, FA and control treatments, the last two being equal.

Regarding reducing sugar, the cultivar with the highest concentration was ‘P’ in relation to ‘B’. Treatments SC and FS increased the reducing sugar content in ‘B’ bulbs when compared to the other treatments. In ‘P’ bulbs, the treatments FA, SC and FS were superior to the control for reducing sugar, being statistically equal to each other.

By analyzing the average values of free total amino acids between cultivars, the cultivar ‘B’ accumulated as much as twice than ‘P’. In addition, the SC and FS treatments in the cultivars average promoted higher accumulated in the content of free total amino acid when compared to the FA and control treatments.

The soluble proteins in the ‘B’ bulbs showed higher concentration when compared to ‘P’. Moreover, in ‘B’, the SC and FS treatments significantly increased the concentration of soluble proteins when compared to FA and control. In ‘P’ bulbs, the treatments showed no statistical differences.

TABLE 4 - EFFECT OF FOLIAR SPRAY WITH FULVIC ACID (FA), *Scenedesmus subspicatus* (SC) AND COMBINATION (FS) TREATMENT ON WEIGHT LOSS (%) OVER 60 DAYS, TOTAL SUGARS, REDUCING SUGAR, FREE AMINO ACIDS AND SOLUBLE PROTEIN OF TWO ONION CULTIVARS CONDUCTED IN THE ORGANIC SYSTEM. VALUES REPRESENT THE MEANS AND ± THE STANDARD DEVIATION. DIFFERENT LETTERS SHOW SIGNIFICANCE (P <0.5) BY THE SCOTT-KNOTT TEST BETWEEN TREATMENTS. CAPITAL LETTERS = TREATMENTS; LOWERCASE LETTERS = CULTIVARS. ANOVA: ns = NOT SIGNIFICANT; * AND ** = SIGNIFICANT AT P ≤ 0.05 AND P ≤0.01, RESPECTIVELY. C = CULTIVARS; T = TREATMENTS AND C X T = INTERACTIONS

Weight Loss (%) over 60 days					
	Control	FA	SC	FS	$\bar{x}$
'B'	16.06 ± 1.80	13.24 ± 2.08	14.28 ± 1.85	12.83 ± 1.88	14.10 a
'P'	14.13 ± 1.62	9.52 ± 2.04	9.74 ± 1.41	10.23 ± 2.55	10.90 b
$\bar{x}$	15.09 a	11.38 b	12.01 b	11.53 b	
ANOVA					
C	**				
T	*				
C x T	ns				
Total Sugars (mg.g^{-1})					
	Control	FA	SC	FS	$\bar{x}$
'B'	21.24 ± 1.28 Ca	19.31 ± 1.79 Ca	24.45 ± 1.47 Aa	22.48 ± 2.25 Ba	21.87 a
'P'	13.24 ± 0.66 Cb	13.07 ± 1.09 Cb	14.97 ± 1.04 Bb	16.95 ± 0.93 Ab	14.56 b
$\bar{x}$	17.24 b	16.19 b	19.71 a	19.72 a	
ANOVA					
C	**				
T	**				
C x T	*				
Reducing Sugar (mg.g^{-1})					
	Control	FA	SC	FS	$\bar{x}$
'B'	23.60 ± 2.39 Bb	21.09 ± 0.68 Bb	27.24 ± 3.54 Ab	30.48 ± 0.52 Ab	25.60 b
'P'	38.56 ± 1.04 Ba	45.73 ± 0.50 Aa	43.65 ± 0.62 Aa	45.79 ± 3.65 Aa	43.43 a
$\bar{x}$	31.08 c	33.41 c	35.44 b	38.14 a	
ANOVA					
C	**				
T	**				
C x T	**				
Free Amino Acids (mg.g^{-1})					
	Control	FA	SC	FS	$\bar{x}$
B'	110.87 ± 5.73	112.93 ± 10.98	130.85 ± 4.99	137.31 ± 12.49	122.99 a
P'	50.32 ± 3.82	51.8 ± 2.97	60.02 ± 3.35	57.48 ± 4.78	54.905 b
$\bar{x}$	80.59 b	82.36 b	95.43 a	97.39 a	
ANOVA					
C	**				
T	**				
C x T	ns				
Soluble Protein (mg.g^{-1})					
	Control	FA	SC	FS	$\bar{x}$
'B'	12.69 ± 1.00 Ba	13.2 ± 0.84 Ba	14.53 ± 0.55 Aa	14.03 ±0.68 Aa	13.61 a
'P'	8.83 ± 0.19 Ab	8.93 ± 0.11 Ab	8.25 ± 0.15 Ab	8.44 ± 0.41 Ab	8.61 b
$\bar{x}$	10.76 a	11.06 a	11.39 a	11.23 a	
ANOVA					
C	**				
T	ns				
C x T	**				

4. DISCUSSION

The bioactivity of the microalgae *Scenedesmus subspicatus* (synonym *Desmodesmus subspicatus*) was found to promote root growth of *V. radiata* plants (Fig. 1ab), a similar result found by Dineshkumar et al. (2018a) using *Chlorella vulgaris* cell extract in *Vigna mung* L. plants. Microalgae have in their composition biomolecules such as proteins, oligosaccharides and phytohormones-like activities molecules capable of promoting plant growth (RONGA et al. 2019).

Similarly, the most reported effect of the application of humic substances on plant growth is related to the change in root architecture, influencing both increase in the length and induction in the lateral root (CANELLAS; OLIVARES, 2014), an effect also observed in the bioassay (Fig. 1cd).

Application of SC, FA and combinations (Fig. 1abcd) promoted root growth and development in *V. radiata*, indicating a potential use as biostimulants. Thus, concentrations to be applied to potted and field plants were chosen based on the best results obtained from the microalgae (1st step) and fulvic acid (2nd step) bioassay, that is, 0.5 gL^{-1} of SC, 0.2 g,L^{-1} of FA, generating the combination (FS) 0.2 $g.L^{-1}$ of FA + 0.5 $g.L^{-1}$ of SC.

Humic substances such as fulvic acid, have the ability to modulate positively the expression of the enzyme H + ATPase in plants, promoting acidification of the plasma membrane, showing as a side effect, the cell expansion without loss of turgor, causing the cell growth (CANELLAS et al. 2015). Similar results were found in a study on onion submitted to foliar applications of humic substances, where they promoted increments in the aerial part of the plants (AL-FRAIHAT et al. 2018). In addition, microalgae biomass contains phytohormones capable of promoting growth in plants such as cytokines and polyamines (STIRK et al. 2013), which stimulates cell division, increasing fresh and dry plant masses (MÓGOR et al. 2018a).

The application of humic acid in chrysanthemum and microalgae in sugar beet resulted in gains in fresh and dry weight with reduction of fresh/dry mass ratio (FAN et al. 2014; MÓGOR et al. 2018b). These results are similar to those found in potted onion plants (Table 1), where the application of FA, SC and FS promoted a reduction in the ratio, and may be related to the higher biomass accumulation of the aerial part when compared to control plant.

Humic substances have the potential to promote growth and development in a variety of plant species, including plants of the Liliaceous family, such as onions

(CANELLAS et al. 2015). Bettoni et al. (2016) when applying HS on onion, observed a significant increase in fresh and dry mass of the bulbs, with equal values of water content in control bulbs, indicating greater accumulation of photoassimilates in the highest priority drains (bulbs), a phenomenon that begins approximately at 90 DAT (VIDIGAL et al. 2010) also coinciding with the start of field applications. This result may partly explain the equal values of the fresh/dry weight ratio in 'P', as there was an increase in the fresh and dry weight of the AF-treated bulbs compared to the control (Fig.2).

Dineshkumar et al. (2018b) report results in increasing the fresh and dry weight of onion plants treated with the microalgae *Spirulina platensis* and *Chlorella vulgaris*. El-sayed et al. (2018) when applying microalgae extract in onion plants observed an increase in fresh and dry weight of bulbs as fresh and dry mass ratio decreased when compared to control, as observed in 'P' where treatments SC and FS promoted increase in the mass, providing a reduction in the fresh/dry mass ratio values, demonstrating a higher biomass accumulation.

Mora et al. (2010) report that the application of HS in plants primarily affects the distribution of nitrate by increasing the activity of H + ATPase, causing concomitantly the distribution of phytohormones that provide plant growth. In addition, an increase in the onion yield has been reported in plants receiving HS as a treatment (BETTONI et al. 2016). Dineshkumar et al (2018b) when applying the biomass of microalgae *Spirulina platensis* and *Chlorella vulgaris* in onion, observed a significant increase in the weight of the treated bulbs, as well as in the productivity and caliber of the bulbs. The increase in productivity may be related to the presence of molecules in microalgae biomass, such as auxin, cytokinin and gibberellin phytohormones that promote growth in the highest priority drains in plants (PLAZA et al. 2018).

According to Oku et al. (2019), the accumulation of fructo-oligosaccharides produced in the aerial part (source) are later translocated to the leaf sheaths to form the bulbs (drains). The application of HS and microalgae promote the increase in carbohydrate production in plants (BETTONI et al. 2016; DINESHKUMAR et al. 2018b), which may cause leaf translocation to bulbs, providing an increase in the caliber.

Zang et al. (2016) observed that out of the carbohydrates found in the dry matter of the onion bulb, non-structural carbohydrates (about 80%) such as glucose, fructose, sucrose and fructo-oligosaccharides are mostly found in it. In addition, Ertani et al. (2011) found that the application of HS in maize plants increased the concentration of

glucose, fructose and the activity of the enzyme rubisco, responsible for carbon fixation in vegetables. This result may be correlated to the increase in carbohydrates (total and reducing sugars) in onions submitted to FA application (Table 3). The use of microalgae in plants shows the potential these microorganisms to promote plant growth and may positively influence carbohydrate concentrations in plants. (GARCIA-GONZALES; SOMERFELD, 2016; EL-SAYED et al. 2018).

In a work testing humic substances from different sources of leonardite, Conselvan et al. (2017) found an increase in enzymes involved in nitrogen metabolism with influences in the increment in amino acid and protein in plants. In addition, microalgae have protein and amino acid content in their composition (TIBBETTS et al. 2015). However, in this study, the application of FA and SC did not clearly show the increase of amino acids and proteins in bulbs of both cultivars.

Studies with different onion cultivars during storage indicate that lower mass losses are correlated with higher dry matter accumulation in the bulbs (KAHSAY et al. 2013), as observed in this study (Table 2) with ‘B’ treated with SC and FS and ‘P’ treated with FA, SC and FS showed an increase in dry mass, which resulted in less bulb loss in storage (Table 4). In addition, the application of humic substances and algae microalgae extract in plants, provided the bulbs in storage lower mass losses in relation to the control, thus, this result was justified by the greater accumulation of dry matter and total soluble solids (SHEHATA et al. 2017; MANSOUR et al. 2019).

Besides the dry mass, the higher carbohydrate accumulation in the bulb contributes to the reduction of mass loss in storage (KAHSAY et al. 2013), as observed, where ‘B’ plants treated with SC and FS and ‘P’ with FA, SC and FS accumulated more sugars, resulting in less mass loss in the bulbs (Tab. 3 and 4). However, for free total amino acids and soluble proteins, it was not possible to clearly identify the effect of greater accumulation of these biomolecules on the bulbs with reduction in mass loss.

5. CONCLUSION

Positive effects on the root architecture of *V. radiata* treated with fulvic acid (FA) and *Scenedesmus subspicatus* microalgae (SC) were detected in the bioassay, with the best results observed in the combination (FS). The effects of foliar applications of solutions containing FA, SC and FS promoted stimulation of growth in the aerial part in the early stage of plant development (up to 60 DAT), with increased field productivity with applications from the beginning of bulbification (90 DAT), which increases the concentration of sugar in the bulbs and proteins in 'B', therefore, reflecting in the reduction of mass losses in storage. However, the application of FS does not potentiate the results in the initial growth, yield and mass loss in both onion cultivars, being the application of SC more efficient.

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6. CAPÍTULO IV – POTENCIAL DA APLICAÇÃO DE MICROALGA ASSOCIADA AO ÁCIDO HÚMICO NA BIOFORTIFICAÇÃO DE CEBOLA ORGÂNICA

Resumo

A biofortificação pode ser entendida como uma estratégia para elevar os níveis de nutrientes nas estruturas comestíveis de vegetais, podendo interferir positivamente na dieta humana. Das diferentes técnicas agronômicas que auxiliam no aumento da qualidade nutricional dos alimentos, o uso de bioestimulantes mostra-se como uma opção interessante no uso em sistemas alternativos de produção, pois são produtos de origem natural que contribuem para sustentabilidade e no desenvolvimento das plantas. A utilização de microalgas e de ácidos húmicos como bioestimulantes apontam para melhorias no teor de nutrientes e de biomoléculas nas plantas, contudo, o emprego de microalgas associado ao ácido húmico ainda é inexplorado. Neste sentido, estudou-se a influência da aplicação via imersão das raízes de mudas de cebola em solução contendo a microalga *Scenedesmus subspicatus* (Sc) e ácido húmico (AH) na alteração de macro e micronutrientes, açúcares totais, açúcar redutor, aminoácidos totais livres, proteínas solúveis e capacidade antioxidante em plantas de cebola. Os tratamentos consistiram na imersão das raízes das mudas de duas cultivares de cebola em soluções contendo a microalga e o ácido húmico por um minuto, sendo posteriormente transplantadas a campo. As concentrações utilizadas foram: controle, 0.3 $g.L^{-1}$ Sc + 0.3 $g.L^{-1}$ AH (3SH) e 0.6 $g.L^{-1}$ Sc + 0.6 $g.L^{-1}$ AH (6SH). Os resultados mostram que a aplicação da microalga associada a ácido húmico foi capaz de incrementar o teor de N, carboidratos e proteínas solúveis, como também aumentar a atividade antioxidante em bulbos de cebola.

Key worlds: *Allium cepa* L.; *Scenedesmus subspicatus*; substâncias húmicas; agricultura orgânica.

1. INTRODUÇÃO

A cebola (*Allium cepa* L.) é originária do continente asiático, sendo uma das espécies hortícolas de importância mundial. Segundo dados da Food and Agriculture Organization (FAO, 2017) a produção mundial de cebolas de 2010 (cerca de 79 milhões de toneladas) a 2017 (97 milhões de toneladas) cresceu cerca de 22%. O maior produtor mundial do bulbo é a China (cerca de 26% da produção mundial) sendo o Brasil ocupante do 9º lugar no ranking. Dada a importância da cebolicultura no Brasil, a atividade encontra-se em 3º lugar no ranking em importância econômica entre as hortaliças, sendo superada pela produção de batata e tomate (KURTZ et al. 2013).

A produção de hortaliças conduzidas em sistema orgânico no mundo mostra-se como uma atividade em ascensão, sendo o Brasil ocupante do 5º lugar no ranking

(MADAIL et al., 2015). A adoção deste sistema de produção vem sendo incentivado pela maior demanda entre os consumidores por alimentos livres de contaminação química e com maior valor nutricional quando comparado com o sistema convencional (BARAŃSKI et al., 2017). Além disso, na comparação do cultivo de cebola em produção orgânica e convencional produzidas por seis anos, Ren et al., (2017) observaram que bulbos produzidos em sistema orgânico apresentaram maior concentração de flavonóides e atividade antioxidante quando comparado a bulbos em sistema convencional.

Os bulbos de cebola apresentam propriedades nutricionais relevantes, sendo ricos em minerais, carboidratos, proteínas e ácido ascórbico (VETHAMONI & GOMATHI, 2018). Além disso, os bulbos também contém moléculas que conferem uma alta capacidade antioxidante, tais como flavonóides quercetina, kaempferol, miricetina e catequina (KARADENIZ et al., 2005).

O'Hare (2015) define o termo biofortificação de plantas como uma prática que promove o aumento dos níveis de nutrientes durante o crescimento e desenvolvimento das partes comestíveis dos vegetais. Neste sentido, tecnologias que possam ser empregadas no sistema orgânico de produção que promovam a biofortificação de alimentos frescos tornam-se interessante.

Estudos mostram que a aplicação de ácido húmico e de extratos de algas em plantas, podem ser consideradas técnicas de biofortificação agronômica, pois apresentam potencial em incrementar o teor de nutrientes e de alterar positivamente a concentração de açúcares totais, aminoácidos, proteínas e compostos fenólicos (BILLARD et al., 2014; CONSELVAN et al., 2017; DINESHKUMAR et al., 2018). Entretanto, o potencial da associação de microalgas com substâncias húmicas ainda é inexplorado. Neste sentido, o objetivo deste trabalho foi explorar o potencial da aplicação da microalga *Scenedesmus subspicatus* e ácido húmico na biofortificação de cebola orgânica.

2. MATERIAL E MÉTODOS

O experimento foi conduzido na área experimental de olericultura orgânica da Universidade Federal do Parará (UFPR), região metropolitana de Curitiba (φ= 25° 25 S, λ= 49° 06′ e 920m de altitude), onde o sistema orgânico vem sendo empregado por mais de uma década. O clima da região de acordo com a classificação de Köppen é o temperado tipo Cfb. A análise química do solo na profundidade de 0-20 cm

identificou os seguintes teores: 6.30 pH (H_2O), 33.30 g.dm^{-3} matéria orgânica; 133.10 mg.dm^{-3} P; 1.44 cmol$_c$.dm^{-3} K; 9.30 cmol$_c$.dm^{-3} Ca; 4.30 cmol$_c$.dm^{-3} Mg; 0 cmol$_c$.dm^{-3} Al; 3.7 cmol$_c$.dm^{-3} Al+H; 18.34 cmol$_c$.dm^{-3} CEC e 80% saturação de bases.

A biomassa da microalga *Scenedesmus subspicatus* (Sc) Chodat (sinônimia: *Desmodesmus subspicatus*), depositada na Coleção de Microalgas "Elizabeth Aidar" da Universidade Federal Fluminense, Niterói, Rio de Janeiro – Brasil, foi obtida em sistema de cultivo autotrófico utilizando meio de cultura WC em temperatura (20-22ºC) e luz (5500 lux) constante, no Departamento de Fitotecnia e Fitossanitarismo da UFPR, Curitiba, Paraná-Brasil. Após 25 dias a biomassa foi centrifugada e liofilizada, estando pronta para o uso. O ácido húmico (HA) provém do mineral Leonardita que contém 33% de carbono orgânico (Powhumus® Humintech GmbH – Germany).

As cultivares de cebolas utilizadas foram a BR-29 ('B') e Perfecta F1 ('P') da empresa Topseed®. As mudas foram produzidas em viveiro tipo túnel coberto com polietileno em canteiros. Após 45 dias após a semeadura (DAS), as mudas foram coletadas para o plantio em canteiro medindo 1.20 x 24 m, sendo distribuídos em parcelas de 1,20 x 1,0 m, em delineamento inteiramente casualizado (n = 4) em esquema fatorial, utilizando um espaçamento entre plantas de 10 cm e entre linhas de 30 cm, resultando em uma população de 230 mil plantas por hectare.

Os tratamentos consistiam em soluções aquosas contendo a microalga (Sc) e o ácido húmico (HA) sendo aplicadas via imersão de raízes das mudas de cebola por um minuto. As concentrações das soluções foram: 0.3 g.L^{-1} Sc + 0.3 g.L^{-1} HA (3SH), 0.6 gL^{-1} Sc + 0.6 gL^{-1} HA (6SH) e água destilada (controle). Aos 150 dias após o transplantio (DAT) das mudas tratadas, cerca de 80% das plantas encontravam-se com a parte aérea tombada rente ao solo (estalo), possibilitando a colheita dos bulbos. Após a colheita, foram amostrados bulbos de cada repetição e cultivar para a realização das análises dos teores de nutrientes, bioquímicas e capacidade antioxidante.

2.1 Análises químicas

Inicialmente foram coletados 2 bulbos por repetição para quantificar teores de macro e micronutrientes (N, K, P, Ca, Mg, Cu, Mn, Fe, Zn e B). Após isso, utilizou-se uma estufa com temperatura (60°C ± 5ºC) e ventilação forçada até as amostras apresentaram peso constante para a obtenção da massa seca de cada amostra. As

amostras com 0,3 g de massa seca de bulbos foram extraídas por digestão ácida conforme descrito por Martins & Reissmann, 2007. Após este procedimento, determinou-se os teores dos nutrientes através do métodos de espectroscopia de emissão óptica de plasma por indução Perkin Elmer Optima 4300 (Perkin Elmer, EUA) em triplicata. A quantificação de nitrogênio (n-total) foi efetuada por combustão em analisador CHONS (modelo Vario EL III).

2.2 Análises bioquímicas

Foram coletados 2 bulbos por repetição de cada cultivar com o objetivo de quantificar açúcares totais, açúcar redutor, aminoácidos totais livres e proteínas solúveis. Para a quantificação dos açúcares totais e redutor preparou-se amostras dos bulbos em microtubos acrescidos de água destilada posteriormente levados em vortex. Após esse processo, os microtubos foram alocados em centrífuga por 10 minutos (10 mil rpm). Para a extração dos açúcares totais adicionou-se em tubos de ensaio a amostra homogeneizada juntamente com 1 ml de HCl para a hidrólise ácida em banho maria por 10 minutos. Em seguida adicionado 1 ml de solução de NaOH deixando-a em repouso por 5 minutos. Após esse procedimento adiciounou-se o reagente DNS nas amostras de açúcares totais e redutor deixando resfriar, sendo possível realizar as leituras em espectrofotômetro a 540 nm. A curva padrão para açúcares redutores e totais foi feita com glicose a 1 mg/mL (5,5 mM) com valores entre 50 a 800 µg/mL (Maldonade et al., 2013).

Para a extração dos aminoácidos livres totais adotou-se a metodologia descrita por Winters et al. (2002), sendo a reação colorimétrica de acordo com com Magné e Larher (1992) utilizando glutamina para a curva padrão. Foram utilizados tudos de ensaio com a amostra vegetal e água destilada para a extração dos aminoácidos, onde foram levados a banho maria a 100°C por 15 minutos, deixando esfriar e decantar a amostra por 20 minutos em banho gelado. Foi retirado o sobrenadante das amostras para microtubos e centrifugadas a 3000 rpm por 10 minutos. A reação colorimétrica foram adicionados 1 ml da amostra, 0,5 ml de tampão citrato, 1ml de ninidrina e agitado por 2 segundos em vortex. Posteriormente utilizou-se banho maria por 15 minutos a 100°C, deixado resfriar, adicionando álcool 60° e realizado as leituras em espectrofotômetro a 570 nm.

A metodologia adotada para a extração das proteínas solúveis foi a descrita por Bradford (1976), sendo para a curva padrão construída utilizando o reagente BSA.

Adicionou-se 0,5 g da amostra vegetal mais 1.5 ml de tampão fosfato como descrito por Du et al. (2010). Após esse procedimento, centrifugou-se a amostra por 15 minutos a 10 mil rpm. Em seguida adicionou-se na amostra homogeneizada 70 µL do sobrenadante mais 2 ml do reagente Bradford descansando por 15 minutos, sendo possível realizar a leitura da amostra em espectrofotômetro a 595 nm.

A ação antioxidante foi estimada usando o padrão 1,1-difenil-2-picril-hidrazil (DPPH), utilizando a metodologia descrita por Brand-Williams et al.(1995). Foram utilizados 0.5g de material vegetal dos bulbos de cebola, diluído posteriormente em água destilada e centrifugados por cinco minutos para a retirada do sobrenadante. Após esse procedimento, foram retirados das amostras 0,1 ml do sobrenadante e transferidos para tubos de ensaio que continham 4,9 ml do reagente DPPH. Em seguida as amostras ficaram em repouso por 40 minutos para aferição em espectrofotômetro a 517nm.

2.3 Análise estatística

O experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial, sendo o fator A composto pelas cultivares (‘B’ e ‘P’) e o fator B os tratamentos (controle, 3SH e 6SH) repetido quatro vezes. Os dados foram submetidos primeiramente ao teste de Bartllet para verificação da homogeneidade das variâncias. Em seguida, realizada a ANOVA utilizando o teste de média Scott-knott a $P \leq 0.05$.

3. RESULTADOS

Em bulbos de ‘P’ (tabela 1) os tratamentos contendo a microalga associada ao ácido húmico não influenciaram no aumento dos teores de nutrientes. Contudo, os tratamentos 3SH e 6SH via imersão incrementaram os teores de N em 17 e 29 % em bulbos de ‘B’ respectivamente, quando comparado com o controle, não apresentando significância para os demais nutrientes. Em comparação entre as cultivares, bulbos de ‘P’ apresentaram na média maior teor de K (18.9 %), Ca (33 %), Mg (19 %), Mn (66.2 %), Fe (296 %) e B (33.8 %) em relação a ‘B’. Em contra partida, bulbos de ‘B’ apresentaram maior concentração de P (15.3 %), Cu (23 %) e Zn (20.4 %). A concentração do mineral nitrogênio, foi na média estatísticamente igual para ambas cultivares.

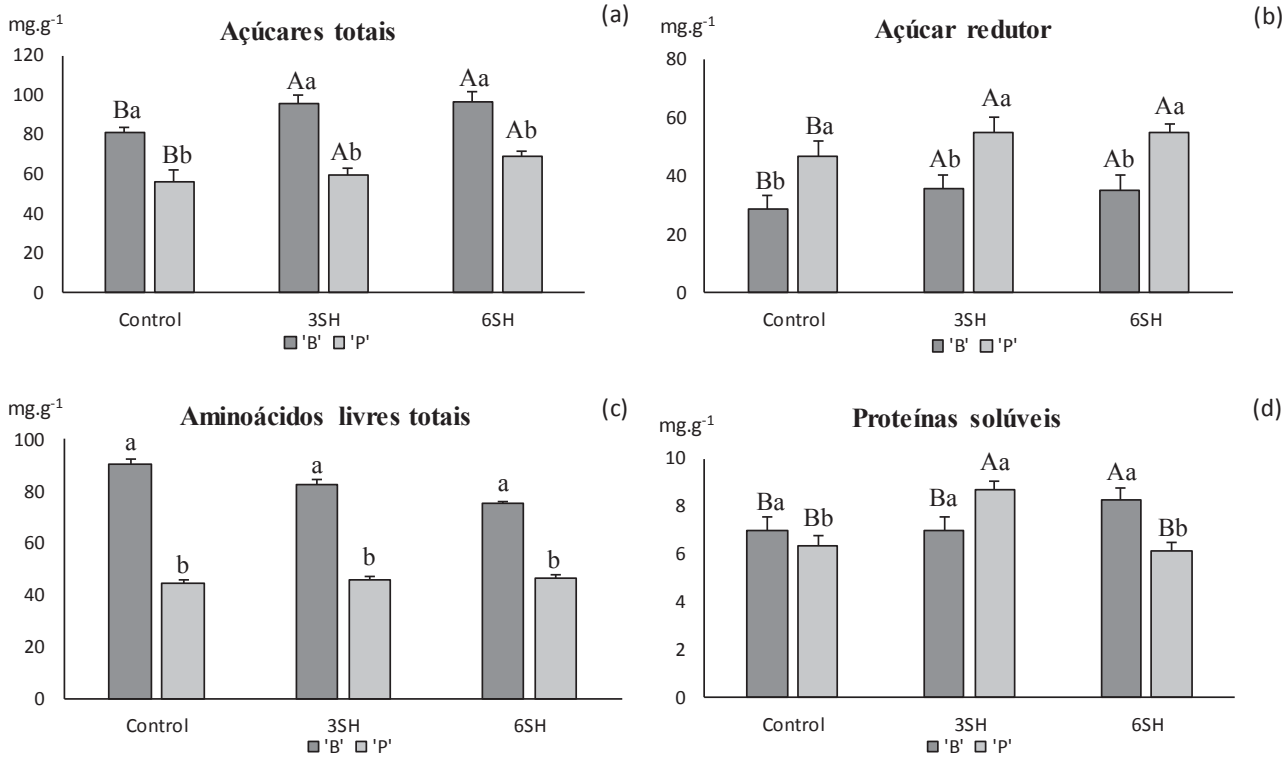
TABELA 1- TEOR DE NUTRIENTES EM BULBOS CEBOLA (MASSA SECA) CULTIVADAS ORGANICAMENTE, CUJAS MUDAS FORAM IMERSAS POR UM MINUTO EM SOLUÇÕES DA BIOMASSA DE MICROALGAS *Scenedesmus subspicatus* (SC) EM ASSOCIAÇÃO AO ÁCIDO HÚMICO (HA). CULTIVARES DE CEBOLAS (P = PERFECTA F1, BR= BR-29). TRATAMENTOS POR IMERSÃO: CONTROLE, 3SH = (0.30 $g.L^{-1}$ SC + 0.30 $g.L^{-1}$ HA), 6SH = (0.60 $g.L^{-1}$ SC + 0.60 $g.L^{-1}$ HA). ANOVA: NS = NÃO SIGNIFICATIVO; * E ** = SIGNIFICATIVO A P ≤ 0.05 AND P ≤0.01, RESPECTIVAMENTE. C = CULTIVARES; T = TRATAMENTOS E C X T = INTERAÇÃO. LETRAS MAIÚSCULAS = TRATAMENTOS POR IMERSÃO.

Treatments		N	P	K	Ca	Mg	Cu	Mn	Fe	Zn	B
				$g.kg^{-1}$					$mg.kg^{-1}$		
B'	Control	14.65 ± 1.45 b	3.95 ± 0.48 a	13.00 ± 0.19 a	2.16 ± 0.16 a	1.43 ± 0.09 a	5.69 ± 0.54 a	5.75 ± 0.63 a	305 ± 19.3 a	24.02 ± 2.01 a	10.91 ± 1.36 a
	3SH	17.18 ± 2.85 a	4.20 ± 0.31 a	13.46 ± 0.65 a	2.06 ± 0.17 a	1.42 ± 0.06 a	5.49 ± 0.79 a	6.86 ± 1.07 a	333 ± 9.60 a	26.20 ± 4.51 a	11.74 ± 1.23 a
	6SH	19.02 ± 2.10 a	4.49 ± 0.32 a	14.09 ± 0.78 a	2.23 ± 0.23 a	1.54 ± 0.21 a	5.63 ± 0.70 a	7.00 ± 1.22 a	305 ± 5.90 a	29.38 ± 2.74 a	11.7 ± 2.09 a
Average ('B')		16.95 A	4.22 A	13.52 B	2.15 B	1.46 B	5.60 A	6.37 B	314 B	26.53 A	11.45 B
P'	Control	15.97 ± 0.30 a	3.37 ± 0.10 a	15.82 ± 0.85 a	2.87 ± 0.44 a	1.74 ± 0.11 a	4.16 ± 0.58 a	9.57 ± 1.47 a	900 ± 70.1 a	20.55 ± 2.22 a	14.15 ± 2.06 a
	3SH	16.35 ± 0.60 a	3.82 ± 0.44 a	16.43 ± 0.92 a	2.88 ± 0.25 a	1.75 ± 0.05 a	4.81 ± 0.45 a	11.38 ± 2.75 a	941 ± 105 a	22.42 ± 2.70 a	16.21 ± 2.03 a
	6SH	15.94 ± 0.38 a	3.79 ± 0.53 a	16.08 ± 0.99 a	2.82 ± 0.19 a	1.73 ± 0.08 a	4.68 ± 0.67 a	10.80 ± 1.93 a	950 ± 145 a	23.10 ± 3.79 a	15.63 ± 2.46 a
Average ('P')		16.09 A	3.66 B	16.08 A	2.86 A	1.74 A	4.55 B	10.59 A	930 A	22.02 B	15.33 A
						ANOVA					
C		ns	*	**	**	**	*	**	**	*	**
T		*	ns	ns	ns	ns	ns	ns	ns	ns	ns
C x T		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Os tratamentos contribuíram para o incremento na concentração de açúcares totais e redutor nos bulbos quando comparado ao controle. Em bulbos de ‘B’ a concentração de açúcares totais (Fig. 1ab) apresentou um acréscimo de 48% quando comparado a bulbos de ‘P’. Enquanto, o conteúdo de açúcar redutor em bulbos de ‘P’ foi significativamente maior (62%) quando comparado a bulbos de ‘B’.

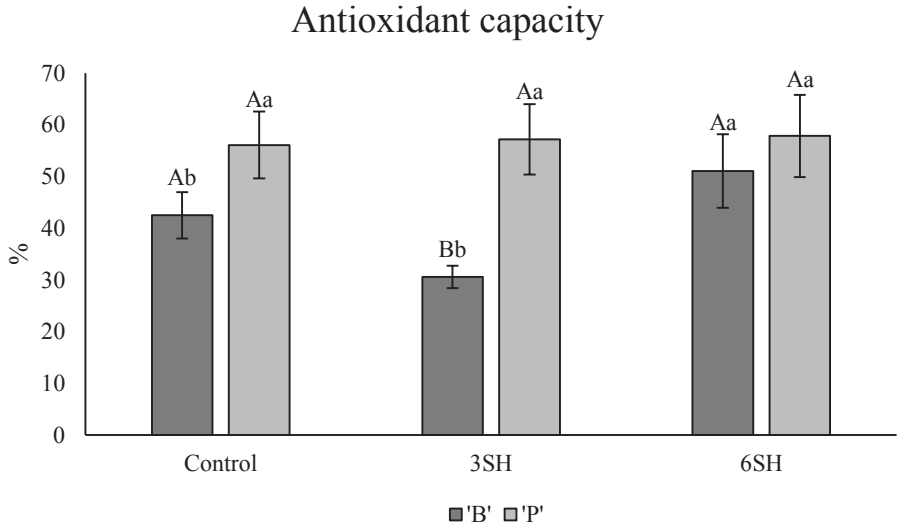
O teor de aminoácidos totais livres (Fig. 1c) em bulbos de ‘B’ foi maior (82%) quando comparado a ‘P’, contudo, sem efeito entre os tratamentos. O teor de proteínas solúveis (Fig. 1d) mostrou interação fatorial entre as cultivares e os tratamentos. A aplicação de 6SH promoveu aumento significativo (18%) em bulbos de ‘B’, sendo em ‘P’, a aplicação de 3SH ganhos significativos de 37% a mais de proteínas solúveis quando comparado ao controle.

FIGURA 1 - CONTEÚDO DE AÇÚCARES TOTAIS (A), AÇÚCAR REDUTOR (B), AMINOÁCIDOS LIVRES TOTAIS (C) E PROTEÍNAS SOLÚVEIS (D) EM BULBOS DE CEBOLA CULTIVADAS ORGANICAMENTE CUJAS MUDAS FORAM IMERSAS POR UM MINUTO EM SOLUÇÕES COM MICROALGA *Scenedesmus subspicatus* BIOMASSA (SC) ASSOCIADA AO ÁCIDO HÚMICO (HA). CULTIVARES DE CEBOLA CINZA ESCURO = BR-29, CINZA CLARO = PERFECTA F1). TRATAMENTOS POR IMERSÃO: CONTROLE, 3SH = (0.30 $g.L^{-1}$ SC + 0.30 $g.L^{-1}$ HA), 6SH = (0.60 $g.L^{-1}$ SC + 0.60 $g.L^{-1}$ HA). COLUNAS COM A MESMA LETRA NÃO DIFERE ESTATÍSTICAMENTE PELO TESTE DE SCOTT-KNOTT (P< 0.01) (N=4). LETRAS MAIÚSCULAS = TRATAMENTOS. LETRAS MINÚSCULAS = CULTIVARES. BARRAS REPRESENTAM DESVIO PADRÃO.



Observou-se diferença significativa na atividade antioxidante entre as cultivares e os tratamentos (Fig. 2). Os bulbos de ‘P’ apresentaram maior capacidade (37%) de sequestrar radicais livres (DPPH) quando comparado aos bulbos de ‘B’. Entre os tratamentos na cultivar ‘P’ não houve diferença estatística. No entanto, na cultivar ‘B’ foi observado maior capacidade antioxidante nos tratamentos controle e 6SH quando comparado ao tratamento 3SH. Além disso, o tratamento 6SH em ‘B’, na média, apresentou capacidade antioxidante estatísticamente igual ao encontrado em bulbos de ‘P’, que apresentaram também maior capacidade antioxidante.

FIGURA 2 - CAPACIDADE ANTIOXIDANTE EM BULBOS DE CEBOLA CULTIVADAS ORGANICAMENTE, CUJAS MUDAS FORAM IMERSAS POR UM MINUTO EM SOLUÇÕES COM MICROALGA *Scenedesmus subspicatus* BIOMASSA (SC) ASSOCIADA AO ÁCIDO HÚMICO (HA). CULTIVARES DE CEBOLA: CINZA ESCURO = BR-29, CINZA CLARO = PERFECTA F1. TRATAMENTOS POR IMERSÃO: CONTROLE, 3SH = (0.30 $g.L^{-1}$ SC + 0.30 $g.L^{-1}$ HA), 6SH = (0.60 $g.L^{-1}$ SC + 0.60 $g.L^{-1}$ HA). COLUNAS COM A MESMA LETRA NÃO DIFERE ESTATÍSTICAMENTE PELO TESTE DE SCOTT-KNOTT (P< 0.01) (N=4). LETRAS MAIÚSCULAS = TRATAMENTOS. LETRAS MINÚSCULAS = CULTIVARES. BARRAS REPRESENTAM DESVIO PADRÃO.



4. DISCUSSÃO

Estudos apontam os potenciais benefícios a saúde humana da cebola como um vegetal fresco, pois considera-se o bulbo com alto valor nutricional, podendo apresentar variação na composição dos nutrientes entre diferentes cultivares (KUMAR et al. 2010). O nitrogênio é um nutriente essencial para o metabolismo humano e das plantas no estímulo da síntese proteica (AL-FRAIHAT, 2009; VIANNA et al.2010). Bettoni et al. (2016) observaram que cerca de 2,8% em média da massa de diferentes cultivares de cebola é composta por nitrogênio, resultado este superior quando comparado aos bulbos de ‘B’ (1,69% de N) e ‘P’ (1,60% de N).

Os tratamentos com 3SH e 6SH proporcionaram aumento do teor de N nos bulbos de ‘B’, podendo este resultado estar relacionado ao efeito do ácido húmico, que quando aplicado em plantas promove a ativação de enzimas envolvidas na redução e assimilação do nitrogênio inorgânico (Nitrato redutase, Nitrito redutase, GS e GOGAT) (VACCARO et al. 2015). Além disso, a biomassa de microalgas pode conter fitohormônios, vitaminas e enzimas que favorecem a maior assimilação de nutrientes em drenos de maior prioridade (SHAABAN et al. 2001).

Dias (2012) aponta que plantas da família das Liliáceas, como cebola e alho, são ricas em potássio, cálcio e manganês, sendo o consumo desses vegetais capazes

de suprir 10% das necessidades diárias humanas. Em 1g de massa fresca de bulbos de cebola, a concentração de potássio e cálcio é respectivamente de 1,46mg e 0,23mg de cálcio (USDA, 2019), dados estes superiores observados nos bulbos de 'B' e 'P' (Tabela 1), sendo o maior teor desses nutrientes quantificados em bulbos da cultivar híbrida em relação a variedade 'B'. O fósforo foi o terceiro nutriente mais acumulado nos bulbos, sendo o magnésio o nutriente com menor teor nos bulbos, resultado semelhante ao encontrado por Bettoni et al. (2016).

Segundo Leão et al. (2018), além da suplementação com ferro em dietas para o combate de anemia, o consumo de alimentos ricos neste micronutriente pode contribuir para elevar a concentração de ferro orgânico na população, reduzindo riscos de deficiência deste mineral. Vidigal et al. (2010) observaram valores médios do teor de Fe em bulbos de cebola de 744 $\mu g.g^{-1}$, concentração esta, diferente do quantificado nas cultivares deste trabalho. Os bulbos de 'P' (930 $\mu g.g^{-1}$) apresentaram na média maior capacidade no acúmulo de Fe em relação a 'B' (314 $\mu g.g^{-1}$), isto mostra que a cultivar híbrida apresenta maior potencial na prevenção de distúrbios nutricionais como a anemia.

Segundo White e Broadley (2005), dentro de uma mesma espécie vegetal há variação na concentração dos elementos químicos, como observado nos genótipos de cebola estudados quando comparamos os micronutrientes Mn, Zn, B e Cu.

Zhang et al., (2016) mostram que na fração da matéria seca de bulbos de cebola, aproximadamente 80% é composta por carboidratos não estruturais, sendo eles açúcares redutores como a glicose e frutose, assim como o açúcar não redutor sacarose e os frutos-oligossacarídeos. Segundo Mallor et al., (2011), os sólidos solúveis estão relacionados ao sabor adocicado da cebola, sendo composto por frutanos, frutose, glicose e sacarose. Estudos com aplicação de ácido húmico em plantas de cebola mostram aumento na concentração de açúcares redutores e amido nos bulbos (BETTONI et al., 2016), assim como a microalga *Nannochloropsis* sp. e *Scenedesmus subspicatus*, que aplicada em plantas de tomate e cebola respectivamente, promoveram aumento dos carboidratos (COPPENS et al., 2016; GEMIN et al. 2019). Foi verificado que os valores de ºBrix nos bulbos das duas cultivares de cebola entre os tratamentos foram iguais ($p > 0.05$). Contudo, bulbos da cultivar 'P' apresentaram maiores valores de ºBrix (44%) quando comparado a bulbos da cultivar 'B'. Os valores médios do ºBrix nos bulbos de 'B' e 'P' são respectivamente: controle = 5.17 e 8.0; 3SH = 6.25 e 8.05; 6SH = 5.57 e 8.45. A contribuição do uso de ácido húmico e microalga como ferramenta em aumentar o conteúdo de

carboidratos nos bulbos é relevante, uma vez que esta tecnologia pode auxiliar os agricultores a obter uma cebola mais doce.

Os bioestimulantes são capazes de promover o aumento de proteínas solúveis em plantas melhorando a assimilação de N e estimulando o metabolismo dos aminoácidos (NARDI et al., 2016). A ação dos tratamentos contendo a microalga associada ao ácido húmico não ficou clara, pois promoveu em 'B' e 'P' maiores acúmulos de proteínas solúveis tratadas com 6SH e 3SH respectivamente, o que não se repetiu nos tratamentos em relação a concentração de aminoácidos livres totais e teor de N em bulbos de 'P' quando tratados com o bioestimulante.

Entre os vegetais, a cebola apresenta alta capacidade antioxidante por conter em sua composição antocianinas e flavonóides, sendo a quercetina o flavonoide com maior concentração em seus bulbos (SULERIA et al. 2015). Ren et al., (2017) observou maior atividade antioxidante e maiores concentrações de flavonóides em cebolas conduzidas em sistema orgânico quando comparado ao convencional, resultado este significante para a produção em sistemas orgânicos e para o consumidor.

Estudos com a aplicação de ácido húmico em plantas demonstram potencial na ativação do metabolismo secundário. Schiavon et al., (2010) mostram que a aplicação de substâncias húmicas aumentaram a expressão da enzima fenilalanina amonialiase, responsável pelo primeiro passo na biossíntese de compostos fenólicos. As microalgas por sua vez, além de apresentarem efeito promotor de crescimento em vegetais, atuam como elicitores no sistema defensivo das plantas, aumentando a concentração de peroxidases, polifenol oxidase e fenilalanina amonialiase, enzimas que atuam como antioxidantes nas plantas (RENUKA et al., 2018). Pode-se observar que a capacidade antioxidante apresenta variações em relação ao genótipo de cebola. Além disso, a aplicação do bioestimulante na concentração de 6SH estimula bulbos de 'B' a atingir uma capacidade antioxidante estatísticamente igual ao observado em bulbos de 'P', genótipo este que apresentou maior capacidade antioxidante.

5. CONCLUSÃO

O uso do biofertilizante contendo a microalga *Scenedesmus subspicatus* em associação com o ácido húmico promoveu aumento do teor de N na cultivar de polinização aberta. Além disso, o biofertilizante proporcionou maior acúmulo de

carboidratos e de proteínas solúveis nos bulbos de ambas cultivares, sendo a capacidade antioxidante aumentada no tratamento 6SH na cultivar BR-29.

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7. CONCLUSÕES GERAIS

A aplicação de substâncias húmicas, tais como, o ácido húmico e fúlvico, assim como da microalga *Scenedesmus subspicatus* indicaram resultados eficientes na promoção do enraizamento de plantas de *Vigna radiata* L. Verificou-se também que a associação dessas substâncias com a microalga potencializaram o desenvolvimento radicular de plantas de *V. radiata*.

No crescimento inicial de plantas de cebola conduzidas em vaso, a associação de ácido húmico e da microalga *Scenedesmus subspicatus* aplicado via imersão de raízes proporcionou ganhos de massa de raízes e da parte aérea. Além disso, o ácido fúlvico, a microalga e sua associação, aplicado via foliar, proporcionaram maior acúmulo de biomassa da parte aérea quando comparado ao controle, sendo o tratamento com a microalga mais eficiente.

A campo, o reflexo dos tratamentos com ácido húmico e da microalga por imersão foram positivos, com aumento das massas dos bulbos e do calibre. Já a aplicação foliar de ácido fúlvico, microalga e associação proporcionou maiores massas de bulbos, com destaque no tratamento com a microalga. Em ambos experimentos a campo observou-se uma interação entre os genótipos e os tratamentos em função da produtividade.

Não só a produtividade foi incrementada, mas também a concentração das variáveis bioquímicas. Os tratamentos via imersão contendo a microalga associado ao ácido húmico proporcionaram aumento nos açúcares totais, redutor, proteínas solúveis e aumento na capacidade antioxidante dos bulbos de cebola. Já a aplicação da associação, bem como separadamente da microalga e ácido fúlvico via foliar em plantas de cebola elevaram as concentrações de açúcares totais, redutor e proteínas solúveis, sendo o tratamento com a microalga mais eficiente.

No armazenamento, os tratamentos contendo substâncias húmicas e microalga foram capazes de reduzir a perda de massa dos bulbos ao longo de 60 dias de armazenamento, proporcionando maiores acúmulos de carboidratos nos bulbos.

8. CONSIDERAÇÕES FINAIS

Pesquisas recentes indicam a potencialidade do uso de microalgas na agricultura como uma alternativa sustentável, de modo a proporcionar ao agricultor ganhos na produtividade. No entanto, futuras investigações devem ser realizadas para identificar o mecanismo de ação das microalgas no metabolismo das plantas, especialmente da microalga *Scenedesmus subspicatus*, que apresentou eficiência na promoção do crescimento vegetal. Recomenda-se também verificar diferentes frequências de aplicação associando-se aos estádios fenológicos das plantas e a persistência do efeito após a aplicação.

A utilização de substâncias húmicas na agricultura é bem difundida, mostrando-se eficiente na aplicação via imersão das raízes e aplicação foliar em cebola, contudo, devem ser conduzidas investigações para estudar diferentes tempos de imersão de raízes de mudas de cebola, bem como em outros cultivos, testando também a persistência após aplicação desses biofertilizantes em plantas.

A associação da microalga *Scenedesmus subspicatus* com substâncias húmicas aplicado em cebola, mostrou-se uma tecnologia benéfica, com potencial para o uso na agricultura. Contudo, a aplicação via foliar apenas com a microalga mostrou-se mais eficiente quando comparada aos demais tratamentos.

Com os resultados obtidos nesta pesquisa, abre-se um leque de possibilidades para testar a associação da microalga com substâncias húmicas em diferentes genótipos de cebola, mais especificadamente nas principais cultivares utilizada entre os agricultores, bem como explorar essa tecnologia em diferentes espécies de cultivos.

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